

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
20 September 2001 (20.09.2001)

PCT

(10) International Publication Number
WO 01/68706 A1

(51) International Patent Classification⁷: **C07K 14/72,**
19/00, C12N 15/62 [US/US]; 126 East Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(21) International Application Number: PCT/US01/08071

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(22) International Filing Date: 14 March 2001 (14.03.2001)

(25) Filing Language:

English (81) Designated States (*national*): CA, JP, US.

(26) Publication Language:

English (84) Designated States (*regional*): European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR).

(30) Priority Data:
60/189,698 15 March 2000 (15.03.2000) US

Published:

— with international search report

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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WO 01/68706 A1

(54) Title: MELANIN CONCENTRATING HORMONE RECEPTOR CHIMERIC AND FUSION PROTEINS

(57) Abstract: The present invention features melanin concentrating hormone receptor (MCH-R) chimeric and fusion proteins. MCH-R chimeric proteins comprise an MCH-R polypeptide region made up of at least two or more polypeptide regions characteristic of MCH-R found in different species. MCH-R fusion proteins comprise an MCH-R polypeptide region and a fluorescent protein region.

TITLE OF THE INVENTION

MELANIN CONCENTRATING HORMONE RECEPTOR CHIMERIC AND
FUSION PROTEINS

5 CROSS-REFERENCE TO RELATED APPLICATIONS

The present application claims priority to provisional application U.S. Serial No. 60/189,698, filed March 15, 2000, hereby incorporated by reference herein.

BACKGROUND OF THE INVENTION

10 The references cited herein are not admitted to be prior art to the claimed invention.

Neuropeptides present in the hypothalamus play a major role in mediating the control of body weight. (Flier *et al.*, 1998. *Cell*, 92, 437-440.) Melanin-concentrating hormone (MCH) is a cyclic 19-amino acid neuropeptide synthesized as 15 part of a larger pre-prohormone precursor in the hypothalamus which also encodes neuropeptides NEI and NGE. (Nahon *et al.*, 1990. *Mol. Endocrinol.* 4, 632-637.) MCH was first identified in salmon pituitary, and in fish MCH affects melanin aggregation thus affecting skin pigmentation. In trout and in eels MCH has also been shown to be involved in stress induced or CRF-stimulated ACTH release. (Kawauchi *et al.*, 1983. *Nature* 305, 321-323.)

20 In humans two genes encoding MCH have been identified that are expressed in the brain. (Breton *et al.*, 1993. *Mol. Brain Res.* 18, 297-310.) In mammals MCH has been localized primarily to neuronal cell bodies of the hypothalamus which are implicated in the control of food intake, including perikarya of the lateral hypothalamus and zona incerta. (Knigge *et al.*, 1996. *Peptides* 17, 1063-25 1073.)

Pharmacological and genetic evidence suggest that the primary mode of MCH action is to promote feeding (orexigenic). MCH mRNA is up regulated in fasted mice and rats and in the *ob/ob* mouse. (Qu *et al.*, 1996. *Nature* 380, 243-247.) 30 Injection of MCH centrally (ICV) stimulates food intake and MCH antagonizes the hypophagic effects seen with α -melanocyte stimulating hormone (α MSH). (Qu *et al.*, 1996. *Nature* 380, 243-247.) MCH-deficient mice are lean, hypophagic, and have increased metabolic rate. (Shimada *et al.*, 1998. *Nature* 396, 670-673.)

35 MCH action is not limited to modulation of food intake as effects on the hypothalamic-pituitary-axis have been reported. (Nahon 1994. *Critical Rev. in*

Neurobiol. 8, 221-262.) MCH may be involved in the body response to stress as MCH can modulate the stress-induced release of CRF from the hypothalamus and ACTH from the pituitary. In addition, MCH neuronal systems may be involved in reproductive or maternal function.

5 Several references describe a receptor that is indicated to bind MCH. (Chambers *et al.*, 1999. *Nature* 400, 261-265; Saito *et al.*, 1999. *Nature* 400, 265-269; Bächner *et al.*, 1999. *FEBS Letters* 457:522-524; Shimomura *et al.*, 1999. *Biochemical and Biophysical Research Communications* 261, 622-626; and Lembo *et al.*, 1999. *Nat. Cell Biol.* 1, 267-271.)

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SUMMARY OF THE INVENTION

15 The present invention features melanin concentrating hormone receptor (MCH-R) chimeric and fusion proteins. MCH-R chimeric proteins comprise an MCH-R polypeptide region made up of at least two or more polypeptide regions characteristic of MCH-R found in different species. MCH-R fusion proteins comprise an MCH-R polypeptide region and a fluorescent protein region.

An MCH-R polypeptide region provides a functional G-protein coupled receptor region able to bind MCH and transduce an intracellular signal. Examples of MCH-R polypeptide regions include naturally occurring MCH-R, 20 chimeric MCH-R containing two or more regions from naturally occurring MCH-R, and functional derivatives thereof.

Reference to the terms "characteristic" and "derivatives thereof" describe a relationship to a reference sequence. In both cases, there is at least about 75% sequence similarity to the reference sequence.

25 Thus, a first aspect of the present invention describes a fusion protein comprising (a) an MCH-R polypeptide region and (b) a fluorescent polypeptide region. The fluorescent polypeptide region is joined directly, or though a polypeptide linker, to the carboxy side of the MCH-R polypeptide region.

Another aspect of the present invention describes an MCH-R chimeric 30 protein. The protein comprises: (a) an MCH-R binding region characteristic of a human MCH-R, (b) a transmembrane domain characteristic of a non-human MCH-R, and (c) an intracellular domain characteristic of a non-human MCH-R.

Another aspect of the present invention describes a nucleic acid 35 encoding for an MCH-R fusion protein or an MCH-R chimeric protein described herein. Such nucleic acid comprises either a contiguous nucleotide sequence that

codes for the protein or a sequence that is processed by a host cell to produce a contiguous nucleotide sequence encoding for the protein. Processing of a nucleic acid sequence to produce a contiguous nucleotide sequence encoding for a protein can occur by the splicing together of exons resulting in intron removal.

5 Another aspect of the present invention describes an expression vector comprising a nucleic acid encoding for an MCH-R fusion protein or an MCH-R chimeric protein described herein.

10 Another aspect of the present invention describes a recombinant cell comprising nucleic acid encoding for an MCH-R fusion protein or an MCH-R chimeric protein described herein. The nucleic acid may be part of the host genome or may exist independently of the host genome.

Another aspect of the present invention describes a non-human transgenic animal comprising nucleic acid encoding for an MCH-R fusion protein or an MCH-R chimeric protein described herein.

15 Another aspect of the present invention describes a method for assaying for MCH-R active compounds by measuring the effect of a test preparation on one or more MCH-R activities. The method is performed using either an MCH-R fusion protein or an MCH-R chimeric protein described herein.

20 Other features and advantages of the present invention are apparent from the additional descriptions provided herein including the different examples. The provided examples illustrate different components and methodology useful in practicing the present invention. The examples do not limit the claimed invention. Based on the present disclosure the skilled artisan can identify and employ other components and methodology useful for practicing the present invention.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates aequorin assay results comparing a mouse MCH-R fusion with a human wild type MCH-R and a CMV-EGFP control.

30 Figure 2 illustrates a cAMP flashplate assay of CHO cell clones stably expressing mMCH-1R-EGFP. Cells from individual clones were dissociated in enzyme free media and stimulated for 15 minutes at 37°C with human MCH at the indicated concentrations in the presence of 10 µM forskolin. Cells were then lysed and assayed for bound [¹²⁵I]cAMP. Mouse MCH-1R-EGFP clones exhibited EC₅₀ values (0.1111, 0.1255, 0.1291, or 0.2304 nM) indistinguishable from that of a CHO cell clone expressing the wild-type human short isoform of MCH-1R (0.1282 nM).

Figure 3 illustrates a cAMP flashplate assay of CHO cell clones stably expressing human short/mouse species chimeric MCH-1R-EGFP. Cells from individual clones were dissociated in enzyme free media and stimulated for 15 minutes at 37°C with human MCH at the indicated concentrations in the presence of 5 10 µM forskolin. Cells were then lysed and assayed for bound [¹²⁵I]cAMP. Human short/mouse species chimeric MCH-1R-EGFP clones exhibited EC₅₀ values (0.0366, 0.0462, 0.2117, or 0.2499 nM) indistinguishable from that of a CHO cell clone expressing the wild-type human short isoform of MCH-1R (0.1137 nM).

10 DETAILED DESCRIPTION OF THE INVENTION

The present invention features MCH-R chimeric and fusion proteins. Such proteins have a variety of different uses including being used as a research tool to study MCH-R function and dynamics, and being used to screen for MCH-R agonists and antagonists.

15 The MCH-R provides a target to achieve different beneficial effects in a patient. Preferably, MCH-R activity is modulated to achieve one or more of the following: weight loss, weight gain, treat cancer (e.g., colon or breast), reduce pain, treat diabetes, reduce stress, or treat sexual dysfunction.

Modulation of MCH-R activity can be achieved by evoking a response 20 at the MCH receptor or by altering a response evoked by an MCH receptor agonist or antagonist. Compounds modulating MCH-R receptor activity include agonists, antagonists, and allosteric modulators. Generally, MCH-R antagonists and allosteric modulators negatively affecting activity will be used to achieve weight loss, treat cancer (e.g., colon or breast), reduce pain, reduce stress, or treat sexual dysfunction; 25 and MCH-R agonists and allosteric modulators positively affecting activity will be used to produce a weight gain.

Preferably, MCH-R activity is modulated to achieve a weight loss or to 30 treat diabetes in a patient. Diabetes mellitus can be treated by modulating MCH-R activity to achieve, for example, one or both of the following: enhancing glucose tolerance or decreasing insulin resistance.

Excessive body weight is a contributing factor to different diseases, including hypertension, diabetes, dyslipidemias, cardiovascular disease, gall stones, 35 osteoarthritis, and certain forms of cancers. Bringing about a weight loss can be used, for example, to reduce the likelihood of such diseases and as part of a treatment for such diseases. Weight reduction can be achieved by modulating MCH-R activity to

obtain, for example, one or more of the following effects: reducing appetite, increasing metabolic rate, reducing fat intake, or reducing carbohydrate craving.

Increasing body weight is particularly useful for a patient having a disease or disorder, or under going a treatment, accompanied by weight loss.

5 Examples of diseases or disorders accompanied by weight loss include anorexia, AIDS, wasting, cachexia, and frail elderly. Examples of treatments accompanied by weight loss include chemotherapy and radiation therapy.

MCH-R Chimeric Proteins

10 MCH-R chimeric proteins contain an MCH-R polypeptide region made up by at least two or more polypeptide regions characteristic of MCH-R found in different species. The different polypeptide regions that are present provide for an N-terminal extracellular domain; a transmembrane domain made up of transmembrane regions, extracellular loop regions, and intracellular loop regions; and an intracellular 15 carboxy terminus domain. Examples of MCH-R amino acid sequences include the following: SEQ. ID. NO. 1 (human MCH1R long form), SEQ. ID. NO. 2 (human MCH1R short form), and SEQ. ID. NO. 3 (mouse MCH1R).

20 Preferably, the MCH-R chimeric protein comprises an MCH-R binding region characteristic of a human MCH-R along with transmembrane and intracellular domains characteristic of a non-human MCH-R. There are substantial amino acid differences between the N-terminus of the MCH-R present in humans and that present in other species such as mice. Such differences could result in, for example, the mouse MCH-R having different intrinsic properties and responsiveness to agonists and/or antagonists than the human MCH-R. The presence of a human MCH-R 25 binding region provides for a "humanized" MCH-R chimeric receptor.

30 The transmembrane and intracellular domains characteristic of a non-human MCH-R can be used in conjunction with a non-human host to provide a more naturally occurring environment for these regions. For example, an MCH-R chimeric having mouse transmembrane and intracellular domains are preferably used in murine cells lines or in transgenic mice.

MCH-R chimeric proteins may contain regions other than extracellular, transmembrane, and intracellular domains that do not substantially decrease the activity of the protein. Preferably, additional regions do not cause a decrease of more than about 25% of MCH-R activity as measured using one or more of the assays

described in the examples provided below. Examples of additional regions that may be present include fluorescent protein regions and linker regions.

In an embodiment of the present invention, the MCH-R chimeric protein comprises: (a) an MCH binding region characteristic of a first species and (b) 5 a transmembrane and intracellular domain region characteristic of a second species joined directly, or though a linker, to the carboxy side of the MCH binding region. Preferably, the protein comprises, consists, or consists essentially of an MCH-R polypeptide having a sequence similarity of at least about 75%, at least 85%, or at least 95% with either SEQ. ID. NO. 4 (human short form/mouse species chimeric 10 MCH1R) or SEQ. ID. NO. 5 (human long form/mouse species chimeric). Even more preferably, the protein comprises, consists essentially of, or consists of, SEQ. ID. NO. 4 or SEQ. ID. NO. 5.

Sequence similarity for polypeptides can be determined by BLAST. (Altschul *et al.*, 1997. *Nucleic Acids Res.* 25, 3389-3402, hereby incorporated by reference herein.) In an embodiment of the present invention, sequence similarity is determined using tBLASTn search program with the following parameters: MATRIX:BLOSUM62, PER RESIDUE GAP COST: 11, and Lambda ratio: 1.

Differences in naturally occurring amino acids are due to different R groups. An R group effects different properties of the amino acid such as physical 20 size, charge, and hydrophobicity. Amino acids can be divided into different groups as follows: neutral and hydrophobic (alanine, valine, leucine, isoleucine, proline, tryptophan, phenylalanine, and methionine); neutral and polar (glycine, serine, threonine, tyrosine, cysteine, asparagine, and glutamine); basic (lysine, arginine, and histidine); and acidic (aspartic acid and glutamic acid).

Generally, in substituting different amino acids it is preferable to exchange amino acids having similar properties. Substituting different amino acids within a particular group, such as substituting valine for leucine, arginine for lysine, and asparagine for glutamine are good candidates for not causing a change in 25 polypeptide functioning.

Changes outside of different amino acids groups can also be made. Preferably, such changes are made taking into account the position of the amino acid 30 to be substituted in the polypeptide. For example, arginine can substitute more freely for nonpolar amino acids in the interior of a polypeptide than glutamate because of its long aliphatic side chain. (See, Ausubel, *Current Protocols in Molecular Biology*, 35 John Wiley, 1987-1998, Supplement 33 Appendix 1C.)

MCH-R Fusion Proteins

MCH-R fusion proteins contain an MCH-R polypeptide region and a fluorescent protein region either directly joined together or joined together through a linker. These regions provide MCH-R activity and a marker for evaluating MCH-R dynamics.

An MCH-R polypeptide region provides functional MCH-R activity and includes naturally occurring MCH-R, chimeric MCH-R, and derivatives thereof. Preferred derivatives thereof have a sequence similarity of at least about 75%, at least 10 about 85%, or at least about 95% to a naturally occurring MCH-R or a chimeric MCH-R described herein.

A fluorescent protein region contains a chromophore that fluoresces. Preferably, the fluorescent protein region is the green fluorescent protein of the jellyfish *Aequorea victoria* or a derivative thereof. Preferred derivatives have a 15 sequence similarity of at least about 75%, at least about 85%, or at least about 95% to the *Aequorea victoria* green fluorescent protein (GFP). The *Aequorea victoria* green fluorescent protein and examples of derivatives thereof are described by Cormack *et al.*, 1996. *Gene* 17, 33-38; Yang *et al.*, 1996. *Nucleic Acids Research* 24, 4592-4593; Tsien *et al.*, U.S. Patent No. 5,625,048; Tsien *et al.*, U.S. Patent No. 5,777,079; and 20 Cormack *et al.*, U.S. Patent No. 5,804,387 (each of which are hereby incorporated by reference herein).

In different embodiments the MCH-R polypeptide region comprises, consists essentially of, or consists of, a sequence selected from the group consisting of: SEQ. ID. NO. 1, SEQ. ID. NO. 2, SEQ. ID. NO. 3, SEQ. ID. NO. 4, and SEQ. ID. 25 NO. 5; and the fluorescent polypeptide region comprises, consists essentially of, or consists of, an amino acid sequence selected from the group consisting of SEQ. ID. NO. 6 (GFP), SEQ. ID. NO. 7 (EGFP), SEQ. ID. NO. 8 (Emerald), SEQ. ID. NO. 9 (Topaz), and SEQ. ID. NO. 10 (W1b). EGFP, Emerald, Topaz, and W1b are derivatives of GFP.

The optionally present linker is a polypeptide region that is preferably from 1 to about 100 amino acids in length. In different embodiments the linker is up to 75, 50 or 25 amino acids in length.

Preferably, the MCH-R fusion protein comprises, consists essentially of, or consists of, the MCH-R polypeptide region and the fluorescent polypeptide 35 region. More preferably, the protein comprises, consists essentially of, or consists of,

an amino acid sequence selected from the group consisting of: SEQ. ID. NO. 11 (mouse MCH1R-linker-EGFP), SEQ. ID. NO. 12 (mouse MCH1R/EGFP direct fusion), SEQ. ID. NO. 13 (human short form/mouse species chimeric MCH1R-linker-EGFP), or SEQ. ID. NO. 14 (human long form/mouse species chimeric MCH1R-linker-EGFP).

MCH-R Chimeric and Fusion Proteins Nucleic Acid and Expression

MCH-R chimeric and fusion proteins can be produced using techniques well known in the art. Preferably, such proteins are produced by recombinant expression inside a host cell by way of an expression vector or by way of nucleic acid integrated into the host genome. Examples of nucleic acid sequences encoding for MCH-R polypeptide regions, fluorescent protein regions, MCH-R chimeric proteins, and MCH-R fusion proteins are provided for by SEQ. ID. NOS. 15-29 (see Example 1, *infra*).

Starting with a particular amino acid sequence and the known degeneracy of the genetic code, a large number of different encoding nucleic acid sequences can be obtained. The degeneracy of the genetic code arises because almost all amino acids are encoded for by different combinations of nucleotide triplets or codons. The translation of a particular codon into a particular amino acid is well known in the art (see, e.g., Lewin GENES IV, p. 119, Oxford University Press, 1990). Amino acids are encoded for by codons as follows:

A=Ala=Alanine: codons GCA, GCC, GCG, GCU
C=Cys=Cysteine: codons UGC, UGU
D=Asp=Aspartic acid: codons GAC, GAU
E=Glu=Glutamic acid: codons GAA, GAG
F=Phe=Phenylalanine: codons UUC, UUU
G=Gly=Glycine: codons GGA, GGC, GGG, GGU
H=His=Histidine: codons CAC, CAU
I=Ile=Isoleucine: codons AUA, AUC, AUU
K=Lys=Lysine: codons AAA, AAG
L=Leu=Leucine: codons UUA, UUG, CUA, CUC, CUG, CUU
M=Met=Methionine: codon AUG
N=Asn=Asparagine: codons AAC, AAU
P=Pro=Proline: codons CCA, CCC, CCG, CCU
Q=Gln=Glutamine: codons CAA, CAG

R=Arg=Arginine: codons AGA, AGG, CGA, CGC, CGG, CGU

S=Ser=Serine: codons AGC, AGU, UCA, UCC, UCG, UCU

T=Thr=Threonine: codons ACA, ACC, ACG, ACU

V=Val=Valine: codons GUA, GUC, GUG, GUU

5 W=Trp=Tryptophan: codon UGG

Y=Tyr=Tyrosine: codons UAC, UAU

Examples of techniques for introducing nucleic acid into a cell and expressing the nucleic acid to produce protein are provided in references such as Ausubel, *Current Protocols in Molecular Biology*, John Wiley, 1987-1998, and

10 Sambrook, *et al.*, in *Molecular Cloning, A Laboratory Manual*, 2nd Edition, Cold Spring Harbor Laboratory Press, 1989.

An expression vector contains recombinant nucleic acid encoding for a polypeptide along with regulatory elements for proper transcription and processing. The recombinant nucleic acid contains two or more nucleic acid regions not naturally 15 associated with each other. Exogenous regulatory elements such as an exogenous promoter can be useful for expressing recombinant nucleic acid in a particular host. Examples of expression vectors are cloning vectors, modified cloning vectors, specifically designed plasmids, and viruses.

Generally, the regulatory elements that are present in an expression 20 vector include a transcriptional promoter, a ribosome binding site, a terminator, and an optionally present operator. Another preferred element is a polyadenylation signal providing for processing in eukaryotic cells. Preferably, an expression vector also contains an origin of replication for autonomous replication in a host cell, a selectable marker, a limited number of useful restriction enzyme sites, and a potential for high 25 copy number.

Expression vectors providing suitable levels of polypeptide expression in different hosts are well known in the art. Mammalian expression vectors well known in the art include pcDNA3 (Invitrogen), pMC1neo (Stratagene), pXT1 (Stratagene), pSG5 (Stratagene), EBO-pSV2-neo (ATCC 37593), pBPV-1(8-2) 30 (ATCC 37110), pdBPV-MMTneo(342-12) (ATCC 37224), pRSVgpt (ATCC 37199), pRSVneo (ATCC 37198), pSV2-dhfr (ATCC 37146), pUCTag (ATCC 37460), pCI-neo (Promega) and .lambda.ZD35 (ATCC 37565). Bacterial expression vectors well known in the art include pET11a (Novagen), lambda gt11 (Invitrogen), pcDNAII (Invitrogen), and pKK223-3 (Pharmacia). Fungal cell expression vectors well known

in the art include pYES2 (Invitrogen) and Pichia expression vector (Invitrogen). Insect cell expression vectors well known in the art include Blue Bac III (Invitrogen).

Recombinant host cells may be prokaryotic or eukaryotic. Examples of recombinant host cells include the following: bacteria such as *E. coli*; fungal cells such as yeast; mammalian cells such as human, bovine, porcine, monkey, hamster, and rodent; and insect cells such as Drosophila and silkworm derived cell lines.

5 Commercially available mammalian cell lines include L cells L-M(TK.sup.-) (ATCC CCL 1.3), L cells L-M (ATCC CCL 1.2), 293 (ATCC CRL 1573), Raji (ATCC CCL 86), CV-1 (ATCC CCL 70), COS-1 (ATCC CRL 1650), COS-7 (ATCC CRL 1651),
10 CHO-K1 (ATCC CCL 61), 3T3 (ATCC CCL 92), NIH/3T3 (ATCC CRL 1658), HeLa (ATCC CCL 2), C127I (ATCC CRL 1616), BS-C-1 (ATCC CCL 26) and MRC-5 (ATCC CCL 171).

15 To enhance expression in a particular host it may be useful to modify the sequence to take into account codon usage of the host. Codon usage of different organisms are well known in the art. (See, Ausubel, *Current Protocols in Molecular Biology*, John Wiley, 1987-1998, Supplement 33 Appendix 1C.)

Expression vectors may be introduced into host cells using standard techniques. Examples of such techniques include transformation, transfection, lipofection, protoplast fusion, and electroporation.

20 Nucleic acid encoding for a polypeptide can be expressed in a cell without the use of an expression vector employing, for example, synthetic mRNA or native mRNA. Additionally, mRNA can be translated in various cell-free systems such as wheat germ extracts and reticulocyte extracts, as well as in cell based systems, such as frog oocytes. Introduction of mRNA into cell based systems can be achieved, 25 for example, by microinjection.

Techniques for producing transgenic animals are well known in the art. Examples of such techniques are provided for by Teratocarcinomas and embryonic stem cells: a practical approach. Ed. By E. J. Robertson, IRL Press Limited, Oxford, England (1987); and Gene Targeting: a practical approach. Ed. By A. L. Joyner,
30 Oxford University Press Inc. New York, NY (1993).

G-Protein Coupled Receptor Assays

MCH-R is G-protein coupled receptor. Techniques for measuring different G-protein activities, such as Gi/o, Gs, and Gq are well known in the art.
35 MCH-R activity is preferably assayed for by measuring either Gi/o or Gq.

Gi/o and Gs activity can be measured using techniques such as a melonaphore assay, measuring cAMP production, measuring inhibition of cAMP accumulation, and measuring binding of ³⁵S-GTP. cAMP can be measured using different techniques such as radioimmunoassay and indirectly by cAMP responsive gene reporter proteins.

5 Gq activity can be measured using techniques such as those measuring intracellular Ca²⁺. Examples of techniques well known in the art that can be employed to measure Ca²⁺ include the use of dyes such as Fura-2 and the use of Ca²⁺-bioluminescent sensitive reporter proteins such as aequorin. An example of a cell line 10 employing aequorin to measure G-protein activity is HEK293/aeq17. (Button *et al.*, 1993. *Cell Calcium* 14, 663-671, and Feighner *et al.*, 1999. *Science* 284, 2184-2188, both of which are hereby incorporated by reference herein.).

15 Functional assays can be performed using individual compounds or preparations containing different compounds. A preparation containing different compounds where one or more compounds affect MCH-R chimeric or fusion protein activity can be divided into smaller groups of compounds to identify the compound(s) affecting MCH-R chimeric or fusion protein activity. In an embodiment of the present invention a test preparation containing at least 10 compounds is used in a functional assay.

20 Functional assays can be performed using recombinantly produced MCH-R chimeric or fusion protein present in different environments. Such environments include, for example, cell extracts and purified cell extracts containing the MCH-R chimeric or fusion protein expressed from recombinant nucleic acid and an appropriate membrane for the polypeptide; and the use of a purified MCH-R 25 chimeric or fusion protein produced by recombinant means that is introduced into a different environment suitable for measuring G-protein activity.

Fluorescent Protein Assays

30 Fluorescent protein joined to an MCH receptor can be employed to study different aspects of receptor dynamics including receptor sequestration, receptor densitization, and receptor localization. The fluorescent protein can be used in *in vitro* or *in vivo* systems.

35 *In vitro* applications of fluorescent proteins can be performed using techniques well known in the art. Examples of such techniques are provided by Barak *et al.*, 1997. *Mol Pharm.* 5, 177-184; Tarasova *et al.*, 1997. *J. Biol. Chem.* 272,

14817-14824; Lin *et al.*, 1998. *Mol. Cell. Endo.* 146, 27-37; Tarasova *et al.*, 1998. *J. Biol. Chem.* 273, 15883-15886; Kallal *et al.*, 1998. *J. Biol. Chem.* 273, 322-328; Groake *et al.*, 1999. *J. Biol. Chem.* 274, 23263-23269; Doherty *et al.*, 1999. *Biochem. J.* 341, 415-422; Brock *et al.*, 1999. *Proc. Natl. Acad. Sci. USA* 96, 10123-10128; Cornea *et al.*, 1999. *Endocrinology* 140, 4272-4280; and Lembo *et al.*, 1999. *Nat. Cell Biol.* 1, 267-271 (these references are not admitted to be prior art to the claimed invention).

In vivo applications of fluorescent proteins can be performed using techniques well known in the art. Examples of such techniques are provided by Mombaerts *et al.*, 1996. *Cell* 87, 675-686; Rodriguez *et al.*, 1999. *Cell* 97, 199-208; Spergel *et al.*, 1999. *J. Neurosci.* 1, 2037-2050; and Zuo *et al.*, 1999. *Proc. Natl. Acad. Sci. USA* 96, 14100-14105 (these references are not admitted to be prior art to the claimed invention).

15 EXAMPLES

Examples are provided below to further illustrate different features and advantages of the present invention. The examples also illustrate useful methodology for practicing the invention. These examples do not limit the claimed invention.

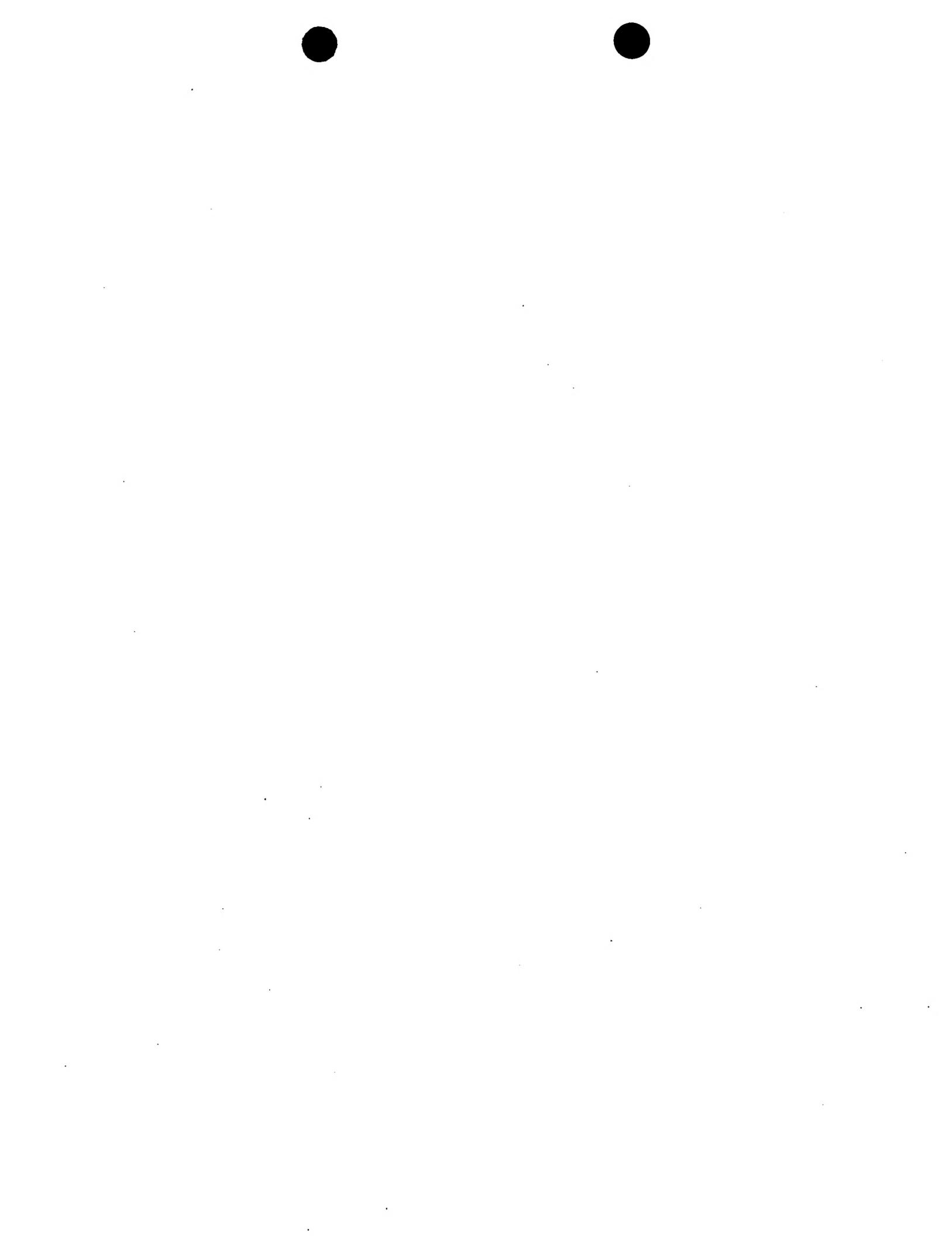
20 Example 1:

Amino acid and nucleic acid sequence information for SEQ. ID. NOs. 1-29 are provided below. SEQ. ID. NOs. 1-29 include examples of polypeptide and encoding nucleic acid sequences for MCH-R polypeptide regions, fluorescent polypeptide regions, fusion proteins and chimeric proteins. In some cases the encoding nucleic acid is shown with additional nucleic acid upstream or downstream from an open reading frame.

SEQ. ID. NO. 1: Human long form MCH1R

MSVGAMKKGVGRAVGLGGGSGCQATEEDPLPNCGACAPGQGGRRWRLPQP
30 AWVEGSSARLWEQATGTGWMDLEASLLPTGPNASNTSDGPDNLTSAGSPPR
TGSISYINIIMPSVFGTICLLGIIGNSTVIFAVVKSKLHWCNNVPDIFINLSVVD
LLFLLGMPFMHQLMNGNVWHFGETMCTLITAMDANSQFTSTYILTAMAIDR
YLATVHPISSTKFRKPSVATLVICLLWALSFISITPVWLYARLIPFPGGAVGCGI
RLPNPDTDLYWFTLYQFFLAFLPFVVITAAYVRILQRMTSSVAPASQRSIRLR





**TKRVTRTAIAICLVFFVCWAPYYVLQLTQLSISRPTLTGVLYNAAISLGYANS
CLNPVFVYIVLCETFRKRLVLSVKPAAQGQLRAVSNAQTADEERTESKGT**

SEQ. ID. NO. 2: Human short form MCH1R

5 MDLEASLLPTGPNASNTSDGPDNLTSAGSPPRTGSISYINIIIMPSVFGTICLLGIIG
NSTVIFAVVKKSKLHWCNNVPDIFIINLSVVDLLFLLGMPFMHQIQLMNGNVWH
FGETMCTLITAMDANSQFTSTYILTAMAIDRYLATVHPISSTKFRKPSVATLVI
CLLWALSFISITPVWLYARLIPFPGGAVGCGIRLPNPDTDLYWFTLYQFFLAFA
LPFVVITAAAYVRILQRMTSSVAPASQRSIRLRTKRVTRTAIAICLVFFVCWAPY
10 YVLQLTQLSISRPTLTGVLYNAAISLGYANSCLNPVFVYIVLCETFRKRLVLSV
KPAAGQQLRAVSNAQTADEERTESKGT

SEQ. ID. NO. 3: Mouse MCH1R

15 MDLQASILLSTGPNASNISDGQDNFTLAGPPPRTRS VS YINIIIMPSVFGTICLLGI
VGNSTVIFAVVKKSKLHWCNSVPDIFIINLSVVDLLFLLGMPFMHQIQLMNGNV
WHFGETMCTLITAMDANSQFTSTYILTAMAIDRYLATVHPISSTKFRKPSMAT
LVICLLWALSFISITPVWLYARLIPFPGGAVGCGIRLPNPDTDLYWFTLYQFFLA
FALPFVVITAAYVKILQRMTSSVAPASQRSIRLRTKRVTRTAIAICLVFFVCWA
PYYVLQLTQLSISRPTLTGVLYNAAISLGYANSCLNPVFVYIVLCETFRKRLVLS
20 VKPAAQGQLRTVSNAQTADEERTESKGT

SEQ. ID. NO. 4: Human short form/mouse species chimeric MCH1R

MDLEASLLPTGPNASNTSDGPDNLTSAGSPPRTGSISYINIIIMPSVFGTICLLGIIG
NSTVIFAVVKKSKLHWCNNVPDIFIINLSVVDLLFLLGMPFMHQIQLMNGNVWH
25 FGETMCTLITAMDANSQFTSTYILTAMAIDRYLATVHPISSTKFRKPSMATLVI
CLLWALSFISITPVWLYARLIPFPGGAVGCGIRLPNPDTDLYWFTLYQFFLAFA
LPFVVITAAYVKILQRMTSSVAPASQRSIRLRTKRVTRTAIAICLVFFVCWAPY
YVLQLTQLSISRPTLTGVLYNAAISLGYANSCLNPVFVYIVLCETFRKRLVLSV
KPAAGQQLRTVSNAQTADEERTESKGT

30

SEQ. ID. NO. 5: Human long form/mouse species chimeric MCH1R

MSVGAMKKGVGRAVGLGGSGCQATEEDPLPNCACAPGQGRRWRLPQP
AWVEGSSARLWEQATGTGWMDEASLLPTGPNASNTSDGPDNLTSAGSPPR
TGSISYINIIIMPSVFGTICLLGIIGNSTVIFAVVKKSKLHWCNNVPDIFIINLSVVD
35 LLFLLGMPFMHQIQLMNGNVWHFGETMCTLITAMDANSQFTSTYILTAMAIDR

YLATVHPISTKFRKPSMATTVICLLWALSFISITPVWLYARLIPFPGGAVGCGI
RLPNPDTDLYWFTLYQFFLAFLALPFVVITAAYVKILQRMTSSVAPASQRSIRLR
TKRVTRTAIAICLVFFVCWAPYYVLQLTQLSISRPLTFVYLYNAAISLGYANS
CLNPFVYIVLCETFRKRLVLSVKPAAQGQLRTVSNAQTADEERTESKGT

5

SEQ. ID. NO. 6: GFP

MSKGEELFTGVVPILVELGDVNNGHKFSVSGEGEGDATYGKLTLKFICTTGKL
PVPWPILVTTFSYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGN
YKTRAEVKFEGLTLVNRIELKGIDFKEDGNILGHKLEYNNSHNVYIMADKQ
10 KNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKD
PNEKRDHMVLEFVTAAGITHGMDELYK

SEQ. ID. NO. 7: EGFP

MVKGEELFTGVVPILVELGDVNNGHKFSVSGEGEGDATYGKLTLKFICTTGK
15 LPVPWPILVTTLYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDG
NYKTRAEVKFEGLTLVNRIELKGIDFKEDGNILGHKLEYNNSHNVYIMADK
QKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSK
DPNEKRDHMVLEFVTAAGITLGMDELYK

20 SEQ. ID. NO. 8: Emerald

Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp
Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr
Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr
Leu Val Thr Thr Leu Thr Tyr Gly Val Gln Cys Phe Ala Arg Tyr Pro Asp His Met Lys
25 Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe
Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr
Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly
His Lys Leu Glu Tyr Asn Ser His Lys Val Tyr Ile Thr Ala Asp Lys Gln Lys
Asn Gly Ile Lys Val Asn Phe Lys Thr Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu
30 Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn
His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met
Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys

SEQ. ID. NO. 9: Topaz

Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp
 Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr
 Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr
 5 Leu Val Thr Thr Phe Gly Tyr Gly Val Gln Cys Phe Ala Arg Tyr Pro Asp His Met Arg
 Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe
 Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr
 Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly
 His Lys Leu Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys
 10 Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu
 Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn
 His Tyr Leu Ser Tyr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met
 Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys

15 SEQ. ID. NO. 10: W1B

Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu Asp
 Gly Asp Val Asn Gly His Arg Phe Ser Val Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr
 Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr
 Leu Val Thr Thr Leu Thr Trp Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
 20 Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile Phe
 Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe Glu Gly Asp Thr
 Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly
 His Lys Leu Glu Tyr Asn Tyr Ile Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys
 Asn Gly Ile Lys Ala His Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu
 25 Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu Leu Pro Asp Asn
 His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met
 Val Leu Leu Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys

SEQ. ID. NO. 11: Mouse MCH1R-linker-EGFP

30 MDLQASLLSTGPNASNISDGQDNFTLAGPPPRTRSVSYINIMPSVFGTICLLGI
 VGNSTVIFAVVKKSKLHWCSNVPDIFIINLSVVDLLFLLGMPFMIHQLMGNGV
 WHFGETMCTLITAMDANSQFTSTYILTAMAIDRYLATVHPISSTKFRKPSMAT
 LVICLLWALSFISITPVWLYARLIPFGGAVGCGIRLPNPDTDLYWFTLYQFFLA
 FALPFVVITAAVKILQRMTSSVAPASQRSIRLRKVRTAIAICLVFFVCWA
 35 PYYVLQLTQLSISRPTLTTFVYLYNAIAISLGYANSCLNPFYIVLCETFRKRLVLS

VKPAAQGQLRTVSNAQTADEEERTESKGTVDGTAGPGSIATMVKGEELFTGV
 VPILVELDGDVNGHKFSVSGEGEGDATYGLTLKFICTTGKLPVPWPTLVTL
 TYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFE
 GDTLVNRIELKGIDFKEDGNILGHKLEYNNNSHNVYIMADKQKNGIKVNFKIR
 5 HNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVL
 LEFVTAAGITLGMDELYK

SEQ. ID. NO. 12: Mouse MCH1R/EGFP direct fusion

MDLQASLLSTGPNASNISDGQDNFTLAGPPRTRSVYINIMPSVFGTICLLGI
 10 VGNSTVIFAVVKSKLHWCSNVPDIFIINLSVVDLLFLLGMPFMIHQLMGNGV
 WHFGETMCTLITAMDANSQFTSTYILTAMAIDRYLATVHPISSTKFRKPSMAT
 LVICLLWALSFISITPVWLYARLIPPGAVGCGIRLPNPDTDLYWFTLYQFFLA
 FALPFVVITAAVKILQRMTSSVAPASQRSIRLRTKRVTRTAIAICLVFFVCWA
 PYYVLQLTQLSISRPTLTIVYLYNAAIISLGYANSCLNPFYIVLCETFRKRLVLS
 15 VKPAAQGQLRTVSNAQTADEEERTESKGTMVKGEELFTGVVPILVELDGDVN
 GHKFSVSGEGEGDATYGLTLKFICTTGKLPVPWPTLVTLTYGVQCFSRYPD
 HMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEKGDTLVNRIELKG
 DFKEDGNILGHKLEYNNNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLAD
 HYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLLEFVTAAGITLG
 20 MDELYK

SEQ. ID. NO. 13: Human short form/mouse species chimeric MCH1R-linker-EGFP

MDLEASLLPTGPNASNTSDGPDNLTSAGSPPRTGSISYINIIMPSVFGTICLLGIIG
 25 NSTVIFAVVKSKLHWCNNVPDIFIINLSVVDLLFLLGMPFMIHQLMGNGVWH
 FGETMCTLITAMDANSQFTSTYILTAMAIDRYLATVHPISSTKFRKPSMATLVI
 CLLWALSFISITPVWLYARLIPPGAVGCGIRLPNPDTDLYWFTLYQFFLAFA
 LPFVVITAAVKILQRMTSSVAPASQRSIRLRTKRVTRTAIAICLVFFVCWAPY
 YVLQLTQLSISRPTLTIVYLYNAAIISLGYANSCLNPFYIVLCETFRKRLVLSV
 30 KPAAQGQLRTVSNAQTADEEERTESKGTVDGTAGPGSIATMVKGEELFTGVV
 PILVELDGDVNGHKFSVSGEGEGDATYGLTLKFICTTGKLPVPWPTLVTTLT
 YGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEKG
 DTLVNRIELKGIDFKEDGNILGHKLEYNNNSHNVYIMADKQKNGIKVNFKIRH
 NIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSKDPNEKRDHMVLL
 35 EFVTAAGITLGMDELYK

SEQ. ID. NO. 14: Human long form/mouse species chimeric MCH1R-linker-EGFP

MSVGAMKKGVGRAVGLGGSGCQATEEDPLPNCACAPGQGGRRWRLPQP
 5 AWVEGSSARLWEQATGTGWMDEASLLPTGPNASNTSDGPDNLTSAGSPPR
 TGSISYINIMPSVFGTICLLGIIGNSTVIFAVVKKSKLHWCNNVPDIFIINLSVVD
 LLFLLGMPFMHQLMNGNVWHFGETMCTLITAMDANSQFTSTYILTAMAIDR
 YLATVHPISSTKFRKPSMATLVICLLWALSFISITPVWLYARLIPFPGGAVGCGI
 RLPNPDTDLYWFTLYQFFLAFLPFVVITAAYVKILQRMTSSVAPASQRSIRLR
 10 TKRVTRTAIAICLVFFVCWAPYYVLQLTQLISRPTLTFVYLYNAAISLGYANS
 CLNPVFVYIVLCETFRKRLVLSVKPAAGQQLRTVSNAQTADEERTESKGTVDGT
 AGPGSIATMVKGEELFTGVVPILEVLDGDVNNGHKFSVSGECEGDAHYGKLT
 LKFICTTGKLPVPWPTLVTTLTGVQCFSRYPDHMKQHDFFKSAMPEGYVQE
 RTIFKDDGNYKTRAEVKFEGLTLVNRIELKGIDFKEDGNILGHKLEYNNSH
 15 NVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHY
 LSTQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYK

SEQ. ID. NO. 15: Human long form MCH1R cDNA

ATGTCAGTGGGAGCCATGAAGAAGGGAGTGGGAGGGCAGTTGGCTTG
 20 GAGGCAGCGGCTGCCAGGCTACGGAGGAAGACCCCCCTCCCAACTGC
 GGGGCTTGCCTCCGGACAAGGTGGCAGGCCCTGGAGGCTGCCGAGC
 CTGCGTGGGTGGAGGGAGCTCAGCTCGGTTGTGGAGCAGGGCACCGG
 CACTGGCTGGATGGACCTGGAAGCCTCGCTGCTGCCACTGGTCCCAACG
 CCAGCAACACCTCTGATGGCCCCGATAACCTCACTTCGGCAGGATCACCT
 25 CCTCGCACGGGAGCATCTCTACATCAACATCATCATGCCCTCGGTGTC
 GGCACCATCTGCCTCCTGGCATCATGGAACTCCACGGTCATCTCGCG
 GTCGTGAAGAAGTCCAAGCTGCACTGGTCAACAACGTCCCCGACATCTT
 CATCATCAACCTCTCGTAGTAGATCTCCTCTTCTCCTGGCATGCCCTT
 CATGATCCACCACTGATGGCAATGGGTGTGGCACTTGGGAGACCA
 30 TGTGCACCCATCACGGCCATGGATGCCAATAGTCAGTTACCCAGCACC
 TACATCCTGACGCCATGGCATTGACCGCTACCTGGCCACTGTCCACCC
 ATCTCTCCACGAAGTCCGGAAGCCCTCTGGCCACCCGGTATCTGC
 CTCCGTGGCCCTCTCCTCATCAGCATACCCCTGTGGCTGTATGCC
 AGACTCATCCCCCTCCCAGGAGGTGCAGTGGCTGCCATACGGCTGCC
 35 CAACCCAGACACTGACCTCTACTGGTCACCCGTACCAAGTTTCCCTGGC

CTTGCCCTGCCCTTGTGGTCATCACAGCCGATACGTGAGGATCCTGCA
GCGCATGACGTCCTCAGTGGCCCCGCCTCCCAGCGCAGCATCCGGCTGC
GGACAAAGAGGGTGACCCGACAGCCATGCCATCTGTCTGGTCTTC
GTGTGCTGGGCACCCTACTATGTGCTACAGCTGACCCAGTTGTCCATCAGC
5 CGCCCGACCCTCACCTTGTCTACTTATAACAATGCGGCCATCAGCTTGGC
TATGCCAACAGCTGCCCTCAACCCCTTGTGTACATCGTGTCTGTGAGACG
TTCCGCAAACGCTTGGTCTGCGGTGAAGCCTGCAGCCCAGGGCAGCT
TCGCGCTGTCAAGCTCAGACGGCTGACGAGGAGAGGACAGAAAGC
AAAGGCACCTGA

10

SEQ. ID. NO. 16: Human short form MCH1R cDNA

ATGGACCTGGAAGCCTCGCTGCTGCCCACTGGTCCCAATGCCAGCAACAC
CTCTGATGGCCCCGATAACCTCACTTCGGCAGGATCACCTCCTCGCACGG
GGAGCATCTCCTACATCAACATCATCATGCCTCGGTGTTGGCACCATCT
15 GCCTCCTGGCATCATCGGAACCTCCACGGTCACTTCGCGGTGTAAG
AAGTCCAAGCTGCACTGGTCAACAACGTCCCCGACATCTTCATCATCAA
CCTCTCGGTAGTAGATCTCCTCTTCTCCTGGCATGCCCTCATGATCCA
CCAGCTCATGGCAATGGGGTGTGGCACTTGGGAGACCATGTGCACCC
TCATCACGGCCATGGATGCCAATAGTCAGTTACCAAGCACCTACATCCTG
20 ACCGCCATGCCATTGACCGCTACCTGGCCACTGTCCACCCATCTCTTCC
ACGAAGTTCCGGAAGCCCTGTGGCCACCCCTGGTGTCTGCCCTGTGG
GCCCTCTCCTCATCAGCATACCCCTGTGTGGCTGTATGCCAGACTCATC
CCCTCCCAGGAGGTGCAGTGGCTGCGGCATACGCCTGCCAACCCAGA
CACTGACCTCTACTGGITCACCTGTACCAAGTTTCTGGCCTTGCCTG
25 CCTTTGTGGTCATCACAGCCGATACGTGAGGATCCTGCAGCGCATGAC
GTCCTCAGTGGCCCCGCCTCCCAGCGCAGCATCCGGCTGCGGACAAAGA
GGGTGACCCGCACAGCCATGCCATCTGTCTGGTCTTGTGTGCTGGG
CACCTACTATGTGCTACAGCTGACCCAGTTGTCCATCAGCCGCCGACCC
TCACCTTGTCTACTTATAACAATGCCATCAGCTTGGCTATGCCAAC
30 GCTGCCTCAACCCCTTGTGTACATCGTGTCTGTGAGACGTTCCGCAAAC
GCTTGGTCTGTGCGGTGAAGCCTGCAGCCCAGGGCAGCTCGCGCTGTC
AGCAACGCTCAGACGGCTGACGAGGAGAGGACAGAAAGCAAAGGCACCT
GA

SEQ. ID. NO. 17: Mouse MCH1R cDNA

Nucleic acid sequence start and stop codons are highlighted:

GGCGGTAGAGGAAGACCCTTTCTGGACTGCCGGGCTCAAGCTCCGGACA
AGGCGGTGGAGGGCGCTGGAGGCTGCCGCAGCCTGCGTGGTGGACGGG
5 CGCTCCACTCCAGGGAGCAGCGACCTGCACCGGCTGCATGGATCTGCAA
GCCTCGTTGCTGTCCACTGGCCCCAATGCCAGAACATCTCCGATGGCCA
GGATAATTTCACATTGGCGGGGCCACCTCCTCGCACAAAGGAGTGTCTCCT
ACATCAACATCATCATGCCTTCAGTGTGGTACCATCTGTCTCCTGGGCA
TTGTGGGAAACTCCACAGTCATTTGCCGTGGTGAAGAAATCCAAGCTG
10 CACTGGTGCAGCAACGTCCTGACATCTCATCATCAACCTCTGTGGTG
GATCTGCTTTCTGCTGGCATGCCTTCATGATCCACCAGCTCATGGGT
AATGGTGTCTGGCACTTGGGAAACCATGTGCACCCATCACAGCCAT
GGACGCCAACAGTCAGTTACCCAGCACCTACATCCTGACTGCTATGGCCA
TTGACCGCTACTTGGCCACCGTCCATCCCATCTCCTCCACCAAGTTCCGGA
15 AGCCCTCCATGCCACCCCTGGTATGCTGCCTCTGTGGCTCTCGTTCA
TTAGCATCACTCCTGTGTGGCTCTATGCCAGGCTTATCCCCTCCCAGGGG
GTGCTGTGGCTGTGGCATCCGCCTACCAAACCCAGATACTGATCTTACT
GGTCACTCTGTATCAGTTTCCTGGCCTCGCCCTCCGTTGTGGTCAT
CACTGCTGCGTACGTGAAAATACTACAGCGCATGACGTCITCGGTGGCCC
20 CAGCCTCTCAACGCAGCATCCGGCTTCGGACAAAGAGGGTGACCCGCACA
GCCATTGCCATCTGTCGGCTTCTTGTGTGCTGGGCCCTACTACGTG
CTGCAGCTGACCCAGTTGTCCATCAGCCGCCACCCACATTGCTCTAC
CTGTACAATGCGGCCATCAGCTGGCTATGCCAACAGCTGCCTCAATCC
CTTGTGTACATAGTACTCTGTGAGACCTTCGAAAACGCTGGTGTGTC
25 GGTGAAGCCCGCGGGCCAGGGCAGCTTCGCACGGTCAGCAATGCTCAGA
CAGCTGACGAGGGAGAGGACAGAAAGCAAAGGCACCTGACAATCCCCCCC
GGTCACCTCCAAGTCAGGTACCGCATCAAACCATGGGGAGAGATACTGA
GATAAACCCGGGCTACCCCTGGAGGATGCAGAAGCTGGAGGCTGGGG
CTTGTAGCAAACCACATTCCACGGGCCACAAATTGCTAGGGAGGCTTG
30 CAGCCTGGTTGGGGGAAGCCTCAGACTGCAGGGATCCCTGACAGA
ATAGAAGCGGAGCAAGAAGGAAAGGGTGGTTGACTGGTCTCGGGGTCT
GTATCTGTTGGCTGCATATATCTTCTCTCAAGGGAAGAAGGCGGAGGT
GCCTAGCTGGGTTCTTAAACTAGGCAGGGCTAGGATCTGAGCAGCTA
GGGCTCTACTGTGAGACTGGCAAGCCGAGCGTCCCATCTCTCATT
35 GGTGTTGATAGAAGGCAGTCTTCTCCAAGCTGGTGGATCTCCTGAAGC

ACGCTGCCTGGGCTCCAGCATCCTGTGCGGATTCACGTTCTTTAGGGG
ATGCATGTTGACACTGGGGTGTGGCTCTGAGGCCACAGGAGTTAAAAAA
ACCAAAAGAGCTCAGAGTGTGAGAGAGACCCAATACCGAGAATGACA
AGGCAACCTGGGGTGGATGTGGATCTGAAACTAATAAAAAGGGGTTTC
5 ACAGTGACAGCGACATTCTCTTCATAGGGCACAGCTGTCACTATGGCT
GATCCAGAGCGAGCATCCATGAATTCTGCATGTGCAGGGGTCACTCTAAT
ACCTGATATGTTGGCATCATCTTGTGCTTGAGCCTCCNCTCCCAAATGG
GAATGAAATAAAGGCAAATTCCNCCCCCCCCCAAAAAAAGGGGNAAAAAA
AAAAAAAAAAAAAAAAAAAAAA

10

SEQ. ID. NO. 18: Mouse MCH1R genomic DNA

Nucleic acid sequence start and stop codons, as well as intron borders, are highlighted:

GGCGGTAGAGGAAGACCCTTTCTGGACTGCGGGCTCAAGCTCCGGACA
15 AGGCGGTGGAGGGCGCTGGAGGCTGCCGAGCCTGCGTGGGTGGACGGG
CGCTCCACTCCAGGGAGCAGGCAGCTGCACCGGCTGCATGGATCTGCAA
GCCTCGTTGCTGTCCACTGGCCCCAATGCCAGCAACATCTCCGATGGCCA
GGATAATTTCACATTGGCGGGTGAGTCGAGTTGGAGTCCTCCCTCCG
GGATGGGTGTGGAAAATGGGAAGGTTCACCTCCAAAGCCAAACTGCCTG
20 GGAAACTTATCTACAGTTGGTGATAAGATCTGCAGTCGGCTTGCC
TGAAGAGGAAGAGGAGAGGAGGGACACCAAGCTAGGACAGAAAGGGCA
GGGAGGAATAGAGATGGGGCAGAGGCACATTAGAAACAACAAGGGTTG
GTGACAAGACGTGAGGCAGGCTTGAGGGAAAGCTTGTGATGAGTCCA
AATATGCTTGCAGGGGGGGGGGGGGGAATCAAGGCTGGAGAAGCAA
25 GCAAGCAAGACAGCAAGACAGCGGGCGGGTAGTATGTGGAGCCAGCAG
AAGCGCTTGTATTACCGCTATCCTGGCTCAATCCTCTGGCTCGCACTG
GGAAATGGGGTCTGAGTGGCTTGCTGTCTCTGGCAAAGGCTGCTGG
GAGCAAAAGACTTCACAGGGCGTGAGAGGATTAACCTTCTGGTGAATT
AGCTTCTTGACATTGCAGAACGTCAATGCCTAAAATTCTAGCTCTGAAG
30 GAGAAGGGAAATGAAGGGAAAGAGGGAAAGGTTGGTGAGAAAATTCCC
AAGCTTCTGGGGTGTAAACACAGCTCCAGTCCTACCCCTATTGGGAAAGCC
CAGACTCAGGAGACATGGTCCAAGGAAATCCCTGACAGAAAACCGGGAG
AGGGCAGGGCTGTGGAGCCTGAAACACACCCCCACACCCATGGTGACAGTC
ACTTCTCACATATGCCTAGGAACCTATCTGAAACCTTGGCCATCTCTC
35 TGAAAAGATGAGGCTGCAAATACACACACACACACACACACACACACAC

ACACACACACACACACACACACACACACACACACACACAAATGTCCITCAAGCC
TTTTGACAAGGTTTCTGGTGGATCCCAGGGATATGAAGTTGTTCTCAGC
AGATATCTGGGAGTCTGACTCCTGGCCCTCTGAGTAAATGGATGAAGCG
AAGAAGAATGGGGTCCTCTGAGTAACAGGTGGATCTAGAAAATCCTATAG
5 GAGTCACCAGGGCACGGTGGAGGAGGGTAAGGTACAGAACTAACATAG
CCCGAGAAGGGAAACAGCAGGAGATGATTCCAGAGACGTAGTGACCCC
AAGCTGCAAGGGAAAGCATGAGGGGCCAGCAGGAAGGCCGACATGGCAG
GTTGTCAGCTCTAGATCGGAAGGCAGGTACACTTGCTCTTCTATCCTC
AGGGCCACCTCCTCGACAAGGAGTGTCTCCTACATCAACATCATCATGC
10 CTTCAGTGTGTTGGTACCATCTGTCTCCTGGCATTGTGGGAAACTCCACAG
TCATTTTGCCTGGTGAAGAAATCCAAGCTGCACTGGTGCAGCAACGTC
CCTGACATCTTCATCATCAACCTCTGTGGTGGATCTGCTTTCTGCTGG
GCATGCCCTTCATGATCCACCAGCTCATGGTAATGGTGTCTGGCACTTG
GGGAAACCATGTGCACCCCATCACAGCCATGGACGCCAACAGTCAGTTC
15 ACCAGCACCTACATCCTGACTGCTATGCCATTGACCGCTACTTGGCCACC
GTCCATCCCATCTCCTCCACCAAGTCCGGAAGGCCCTCCATGCCACCCCTG
GTGATCTGCCCTCTGTGGCTCTCGTCATTAGCATCACTCCTGTGTGG
CTCTATGCCAGGCTTATCCCCCTCCAGGGGGTGCTGTGGGCTGTGGCATIC
CGCCTACCAAACCCAGATACTGATCTTACTGGITCACTCTGTATCAGTT
20 TTCCTGGCCTTCGCCCTCCGTTGTGGTCATCACTGCTGCGTACGTAAA
ATACTACAGCGCATGACGTCTCGTGGCCCCAGCCTCTCAACGCAGCAT
CCGGCTTCGGACAAAGAGGGTGACCCGCACAGCCATTGCCATCTGTCTGG
TCTCTTGTGTGCTGGCGCCCTACTACGTGCTGCAGCTGACCGAGTTGT
CCATCAGCCGCCGACCCCTCACATTGCTTACCTGTACAATGCCGCATCA
25 GCTTGGCTATGCCAACAGCTGCCATCCCTTGTGTACATAGTACTCT
GTGAGACCTTCGAAAACGCTTGGTGTGTCGGTAAGCCCGGGCCAG
GGCAGCTCGCACGGTCAGCAATGCTCAGACAGCTGACGAGGAGAGGA
CAGAAAGCAAAGGCACCTGACAATCCCCCGGTACCTCCAAGTCAGGT
CACCGCATCAAACCATGGGAGAGATACTGAGATAAACCCGGGCTACC
30 CTGGGAGGATGCAGAAGCTGGAGGCTGGGGCTTGTAGCAAACACATTG
CACGGGGCCCACAAATTGCTAGGGAGGCTTGCAGCCTGGTTGGGGGGGA
AGCCTCAGACTGCAGGGATCCCTGACAGAATAGAAGCGGAGCAAGAA
GGAAAGGGTGGTTGACTGGTCTCGGGTCTGTATCTGTTGGCTCGCATA
TATCTTCTCAAGGAAAGAAGGCGGAGGTGCCTAGCTGGTTCCCTTA
35 AAACTAGGCAGGGCTAGGATCTGAGCAGCTAGGGCTACTGTGAGACTG

GGCAAGCCGAGCGTCCCTCCCATCTCTCATTGGTGTGATAGAAGGCAG
TCTTCCTCCCAAGCTGGTGGATCTCCTGAAGCACGCTGCCCTGGGCTCCAGC
ATCCTGTGCGGATTCACGTTCTCTTAGGGATGCATGTTGACACTGGGG
TGTGGGCTCTGAGCCCACAGGAGTTAAAAAACCAAAAGAGCTCAGAGTG
5 TCGAGAGAGACCCAATCACCGAGAATGACAAGGCAACCTGGGTGGATG
TGGATCTGAAACTAATAAAAAGGGGTTTCACAGTGACAGCGACATTCT
CTTCATAGGGCACAGCTGTCACTATGGCTGATCCAGAGCGAGCATCCA
TGAATTCTGCATGTGCAGGGTCACTCTAATACCTGATATGTTGGCATCAT
CTTGTGCTTGAGCCTCCNCTCCAAATGGGAATGAAATAAAGGCAAAT
10 TCCCNCCCCCCCCAAAAAAAGGGNAAAAAAAAAAAAAAAAAAAAAAA
AA

SEQ. ID. NO. 19: Human short form/mouse species chimeric MCH1R

ATGGACCTGGAAGCCTCGCTGCTGCCACTGGTCCAATGCCAGCAACAC
15 CTCTGATGGCCCCGATAACCTCACTTCGGCAGGATCACCTCCTCGCACGG
GGAGCATCTCCTACATCAACATCATCATGCCTCGGTGTCGGCACCATCT
GCCTCCTGGGCATCATGGGAACTCCACGGTCATCTCGCGGTGTAAG
AAGTCCAAGCTGCACTGGTCAACAAACGTCCCCGACATCTCATCAA
CCTCTCGTAGTAGATCTCCTCTTCTCCTGGCATGCCCTCATGATCCA
20 CCAGCTCATGGCAATGGGTGTGGCACTTGGGAGACCATGTGCACCC
TCATCACGGCCATGGATGCCAATAGTCAGTTACCCAGCACCTACATCCTG
ACGCCATGGCATTGACCGCTACCTGGCCACTGTCCACCCATCTCTCC
ACGAAGTTCCGGAAGCCCTCCATGCCACCCCTGGTATCTGCCCTGTG
GGCTCTCGITCATTAGCATCACTCCTGTGTGGCTATGCCAGGCTTAT
25 CCCCTCCCAGGGGTGCTGTGGCTGTGGCATCCGCCTACCAAACCCAG
ATACTGATCTTACTGGTCACTCTGTATCAGTTCTGGCCTCGCCCT
TCCGTTGTGGTCATCACTGCTGCGTACGTGAAAATACTACAGCGCATGAC
GTCTCGGTGGCCCCAGCCTCTCAACGCAGCATCCGGCTCGGACAAAGA
GGGTGACCCGCACGCCATTGCCATCTGTCTGGCTTCTTGTGTGCTGGG
30 CGCCCTACTACGTGCTGCAGCTGACCCAGTTGTCCATCAGCCGCCGACC
CTCACATTGCTCACCTGTACAATGCCGCATCAGCTGGCTATGCCAAC
AGCTGCCTCAATCCCTTGTGTACATAGTACTCTGTGAGACCTTCGAAAA
CGCTTGGTGTGTCGGTGAAGCCCGCGCCAGGGCAGCTCGCACGGT
CAGCAATGCTCAGACAGCTGACGAGGAGAGGACAGAAAGCAAAGGCACC
35 TGA

SEQ. ID. NO. 20: Human long form/mouse species chimeric MCH1R

ATGTCAGTGGGAGCCATGAAGAAGGGAGTGGGGAGGCAGTTGGGCTTG
GAGGCGGCAGCGGCTGCCAGGCTACGGAGGAAGACCCCCCTCCCAACTGC
5 GGGGCTTGCCTCCGGACAAGGTGGCAGGCCTGGAGGGCTGCCGAGC
CTGCGTGGGTGGAGGGAGCTCAGCTCGTTGTGGAGCAGGCGACCGG
CACTGGCTGGATGGACCTGGAAGCCTCGCTGCTGCCACTGGTCCAACG
CCAGCAACACCTCTGATGGCCCCGATAACCTCACITCGGCAGGATCACCT
CCTCGCACGGGGAGCATCTCCTACATCAACATCATCATGCCCTCGGTGTT
10 GGCACCACATCTGCCTCCTGGGCATCATCGGAACACTCCACGGTCATCTTCGCG
GTCGTGAAGAAGTCCAAGCTGCACTGGTCAACAACGTCCCCGACATCTT
CATCATCAACCTCTCGTAGTAGATCTCCTTTCTCCTGGCATGCCCTT
CATGATCCACCAGCTCATGGCAATGGGTGTGGCACTTGGGAGACCA
TGTGCACCCTCATACGGCCATGGATGCCAATAGTCAGTTACCAGCACC
15 TACATCCTGACCGCCATGCCATTGACCGCTACCTGGCCACTGTCCACCCC
ATCTCTTCCACGAAGTCCGGAAGCCCTCCATGCCACCCCTGGTATCTGC
CTCCTGTGGCTCTCGTTAGCATCACTCCTGTGTGGCTATGCC
AGGCTTATCCCCCTCCCAGGGGGTGTGTGGCTGTGGCATCCGCCTACCA
AACCCAGATACTGATCTTACTGGTCACTCTGTATCAGTTTCCCTGGCCT
20 TCGCCCTCCGTTGTGGTCATCACTGCTGCGTACGTGAAAATACTACAGC
GCATGACGTCTCGGTGGCCCCAGCCTCTAACGCAGCAGCATCCGGCTTCGG
ACAAAGAGGGTGACCCGCACAGCCATTGCCATCTGTCTGGCTTCTTGTG
TGCTGGCGCCCTACTACGTGCTGCAGCTGACCCAGTTGTCCATCAGCCGC
CCGACCCCTCACATTGCTCACCTGTACAATGCCATCAGCTTGGGCTAT
25 GCCAACAGCTGCCCAATCCCCTTGTGTACATAGTACTCTGTGAGACCTT
CGAAAACGCTTGGTGTGTCGGTGAAGCCCGGGCCAGGGGCAGCTTCG
CACGGTCAGCAATGCTCAGACAGCTGACGAGGAGAGGACAGAAAGCAA
GGCACCTGA

30 SEQ. ID. NO. 21: *Aequorea victoria* Green Fluorescent Protein (GFP) cDNA

Nucleic acid sequence start and stop codons are highlighted:

TACACACGAATAAAAGATAACAAAGATGAGTAAAGGAGAAGAACCTTTC
ACTGGAGTTGTCCTAATTCTGTTGAATTAGATGGTGATGTTAATGGGCAC
AAATTTCTGTCAGTGGAGAGGGTGAAGGTGATGCAACATACGGAAAAC
35 TACCCCTAAATTATTCGCACTACTGGAAAACCTACCTGTTCCATGGCCAAC

ACTTGTCACTACTTCTCTATGGTGTCAATGCTTTCAAGATAACCCAGAT
CATATGAAACAGCATGACTTTCAAGAGTGCATGCCGAAGGTTATGT
ACAGGAAAGAACTATATTTCAAAGATGACGGAACTACAAGACACGTG
CTGAAGTCAAGTTGAAGGTGATACCCTGTTAATAGAATCGAGTTAAAA
5 GGTATTGATTTAAAGAAGATGGAAACATTCTGGACACAAATTGGAATA
CAACTATAACTCACACAATGTATACATCATGGCAGACAAACAAAAGAATG
GAATCAAAGTTAACCTCAAAATTAGACACAACATTGAAGATGGAAGCGTT
CAACTAGCAGACCATTATCAACAAATACTCCAATTGGCGATGCCCTGT
CCTTTACCAAGACAACCATTACCTGTCCACACAATCTGCCCTTCGAAAGA
10 TCCCAACGAAAAGAGAGACCACATGGCCTCTTGAGTTGTAACAGCTG
CTGGGATTACACATGGCATGGATGAACTATACAAATAATGTCCAGACTT
CCAATTGACACTAAAGTGTCCGAACAATTACTAAAATCTCAGGGTTCTG
GTAAATTCAAGGCTGAGATATTATATATTATAGATTCAATTAAACATT
GTATGAATAATTATTGATGTTATTGATAGAGGTTATTCTTATTAAACA
15 GGCTACTTGGAGTGTATTCTTAATTCTATATTAATTACAATTGATTGACT
TGCTAAA

SEQ. ID. NO. 22: EGFP + Linker

Nucleic acid sequence start and stop codons are highlighted and a 12 amino acid
20 linker sequence is denoted in lower case:
gtcgacggtaaccgcgggccccggatccatgcacc**ATGGTGAGCAAGGGCGAGGAGCTGTT**
CACCGGGGTGGTCCCCATCCTGGTCAGCTGGACGGCGACGTAAACGGCC
ACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAA
GCTGACCCTGAAGTTCATCTGCACCACCGCAAGCTGCCGTGCCCTGGC
25 CCACCCCTCGTGACCACCCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTAC
CCCACCATGAAGCAGCAGCAGTCTCAAGTCCGCCATGCCGAAGG
CTACGTCCAGGAGCGCACCATCTTCTCAAGGACGACGGCAACTACAAGA
CCCGCGCCGAGGTGAAGTTGAGGGCGACACCCCTGGTGAACCGCATCGAG
CTGAAGGGCATCGACTCAAGGAGGACGGCAACATCCTGGGGCACAAGC
30 TGGAGTACAACACAAACAGCCACAACGTCTATATCATGGCCGACAAGCAG
AAGAACGGCATCAAGGTGAACCTCAAGATCCGCCACAACATCGAGGACG
GCAGCGTGCAGCTGCCGACCAACTACCAGCAGAACACCCCCATCGCGAC
GGCCCCGTGCTGCCGACAACCAACTACCTGAGCACCCAGTCCGCCCT
GAGCAAAGACCCCAACGAGAAGCGCGATCACATGGCCTGCTGGAGTTCG

TGACCGCCGCCGGGATCACTCTGGCATGGACGAGCTGTACAAGTAAAGC
GGCCGC

SEQ. ID. NO. 23: Emerald

5 ATGGTGAGCAAGGGCGAGGAGCTGTTACCAGGGTGGTGCCTACCTGGT
CGAGCTGGACGGCGACGTAAACGCCACAAGTTAGCGTGTCCGGCGAG
GGCGAGGGCGATGCCACCTACGGCAAGCTGACCCCTGAAGTTCATCTGCAC
CACCGGCAAGCTGCCGTGCCCTGGCCCACCCCTCGTGACCACCTGACCT
ACGGCGTGCAGTGCTCGCCCGTACCCCGACCACATGAAGCAGCACGAC
10 TTCTCAAGTCCGCCATGCCGAAGGCTACGTCCAGGAGCGCACCATCTTC
TTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGG
GCGACACCCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAG
GACGGCAACATCCTGGGGACAAGCTGGAGTACAACATACAACAGCCACA
AGGTCTATATCACCGCCGACAAGCAGAAGAACGGCATCAAGGTGAACCTC
15 AAGACCCGCCACAACATCGAGGACGGCAGCGTGCAGCTGCCGACCACT
ACCAGCAGAACACCCCCATCGCGACGGCCCGTGTGCTGCCGACAAC
CACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCAACGAGAACCG
CGATCACATGGTCTGCTGGAGTTCGTGACCGCCGGGATCACTCTCG
GCATGGACGAGCTGTACAAGTAA

20

SEQ. ID. NO. 24: Topaz

ATGGTGAGCAAGGGCGAGGAGCTGTTACCAGGGTGGTGCCTACCTGGT
CGAGCTGGACGGCGACGTAAACGCCACAAGTTAGCGTGTCCGGCGAG
GGCGAGGGCGATGCCACCTACGGCAAGCTGACCCCTGAAGTTCATCTGCAC
25 CACCGGCAAGCTGCCGTGCCCTGGCCCACCCCTCGTGACCACCTCGGCT
ACGGCGTGCAGTGCTCGCCCGTACCCCGACCACATCGGCCAGCACGAC
TTCTCAAGTCCGCCATGCCGAAGGCTACGTCCAGGAGCGCACCATCTTC
TTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGG
GCGACACCCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAG
30 GACGGCAACATCCTGGGGACAAGCTGGAGTACAACATACAACAGCCACA
ACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACCTC
AAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTGCCGACCACTA
CCAGCAGAACACCCCCATCGCGACGGCCCGTGTGCTGCCGACAACC
ACTACCTGAGCTACCAGTCCGCCCTGAGCAAAGACCCAACGAGAACCGC

GATCACATGGCCTGCTGGAGTCGTACCGCCGCCGGGATCACTCTCGG
CATGGACGAGCTGTACAAGTAA

SEQ. ID. NO. 25: W1B

5 ATGGTGAGCAAGGGCGAGGAGCTGTTACCGGGTGGTGCCTACCTGGT
CGAGCTGGACGGCGACGTAAACGCCACAGGTCAGCGTGTCCGGCGAG
GGCGAGGGCGATGCCACCTACGGCAAGCTGACCTGAAGTTCATCTGCAC
CACCGGCAAGCTGCCGTGCCCTGGCCCACCCTCGTGACCACCGTACCT
GGGGCGTGCAGTGCTCAGCCGCTACCCGACCACATGAAGCAGCACGAC
10 TTCTTCAAGTCCGCCATGCCGAAGGCTACGTCCAGGAGCGTACCATCTTC
TTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGG
GCGACACCCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAG
GACGGCAACATCCTGGGGACAAGCTGGAGTACAACATACATCAGCCACA
ACGTCTATATCACCGCCGACAAGCAGAAGAACGGCATCAAGGCCACTTC
15 AAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTA
CCAGCAGAACACCCCCATCGCGACGGCCCCGTGCTGCTGCCGACAACC
ACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCAACGAGAAGCCG
GATCACATGGCCTGCTGGAGTCGTACCGCCGCCGGATCACTCTCGG
CATGGACGAGCTGTACAAGTAA

20

SEQ. ID. NO. 26: Mouse MCH1R-linker-EGFP

Nucleic acid sequence start codon and start and stop codons for mouse MCH1R and EGFP, respectively, as well as intron borders, are highlighted and a 12 amino acid linker sequence is denoted in lower case:

25 ATGGATCTGCAAGCCTCGTGTCCACTGGCCCCAATGCCAGCAACAT
CTCCGATGGCCAGGATAATTACATTGGCGGGTGAGTCGAGTTGGAGTC
CTCCCTCCTCCGGGATGGGTGTGGAAAATGGGAAGGTTCACCTCCAAG
CCAAACTGCCTGGAAACTTATCTTACAGTTCTGGTATAAGATCTGCA
GTCGGCTTGCTGAAGAGGAAGAGGAGAGGAGGGACACCAGCTAGGA
30 CAGAAGGGCAGGGAGGAATAGAGATGGGCAGAGGCACATTAGAAAC
AACAAAGGGTTGGTGACAAGACGTGAGGCAGGCTGAGGGAAAGCTTGC
TGATGAGTCCAAATATGCTTGCAGGGGGGGGGGGGGGGAAATCAAGG
CTGGAGAAGCAAGCAAGACAGCAAGACAGCAGCGGGCGGGTAGTATGT
GGGAGCCAGCAGAACCGCTTGATTACCGCTATCCTGGCTCAATCCTC
35 TGGCCTCGCACTGGGAAATGGGTCTGAGTGGTCTGCTGTCTCTGGC

AAAGGCTGCTGGAGCAAAAGACTCACAGGGCGTGAGAGGGATTAACCTT
TCTGGTGAATTAAAGCTTCTGACATTGCAGAACGTCAATGCCTAAAATT
CTAGCTCTGAAGGAGAAGGGAAATGAAGGGGAAAGAGGGAAAGGTTGGTGT
GGAGAAATTCCAAGCTCTGGGTGTAAACACAGCTCCAGTCCTACCCCT
5 ATGGGAAAGCCCAGACTCAGGAGACATGGTCCAAGGAAATCCCTGACA
GAAAACCGGGAGAGGGCAGGGCTGTGGAGCCTGAAACACACACCCACACC
CATGGTACAGTCACCTCTCACATATGCCTAGGAACCTATCTGAAACCTT
GCCATCTCTCTGAAAAGATGAGGCTGCAAATACACACACACACACAC
ACAAA
10 TGTCTTCAAGCCTTTGACAAGGTTCTGGTGGATCCGGGATATGA
AGTTGTTCTCAGCAGATATCTGGAGTCTGACTCCTGGCCCTCTGAGTAA
ATGGATGAAGCGAAGAAGAATGGGTCCTCTGAGTAACAGGTGGATCTA
GAAAATCCTATAGGAGTCACCAGGGCACGGTGGAGGAGGGTAAGGTACA
GAACTAACAATAGCCCAGAGAAGGGAAACAGCAGGAGATGATTCCAGAG
15 ACGTAGTGACCCCCAAGCTGCAAGGGAAAGCATGAGGGCCAGCAGGAAG
GCCGACATGGCAGGTGTCAGCTCTAGATCGGAAGGGGGTACACTTG
CTCTTCTATCCTCAGGGCACCTCTCGACAAGGAGTGTCTCCTGGCATTGTG
AACATCATCATGCCCTCAGTGTGGTACCATCTGTCTCCTGGCATTGTG
GGAAACTCCACAGTCATTITGCCGTGGTGAAGAAATCCAAGCTGACTG
20 GTGCAGCAACGTCCCTGACATCTTCATCATCAACCTCTGTGGTGGATCT
GCTTTCTGCTGGCATGCCCTCATGATCCACAGCTCATGGTAATGG
TGTCTGGCACTTGGGAAACCATGTGCACCCATCACAGCCATGGACCG
CCAACAGTCAGTCACCAGCACCTACATCCTGACTGCTATGCCATTGACC
GCTACTTGGCCACCGTCCATCCCATCTCCTCCACCAAGTCCGGAAGCCCT
25 CCATGGCCACCCCTGGTATGCTGCCCTGTGGCTCTCGTTCTAGCA
TCACTCCTGTGTGGCTCTATGCCAGGGCTATCCCTCCAGGGGGTGTG
TGGCTGTGGCATCCGCTACCAAAACCCAGATACTGATCTTACTGGTCA
CTCTGTATCAGTTTCTGGCTTCGCCCTCCGTTGTGGTATCACTGC
TGCGTACGTGAAAATACTACAGCGCATGACGTCCTCGGTGGCCCCAGCCT
30 CTCAACCGCAGCATCCGGCTCGGACAAAGAGGGTACCCGCACAGCCATT
GCCATCTGTCTGGCTTCTTGTGTGCTGGCGCCCTACTACGTGCTGCAG
CTGACCCAGTTGTCCATCAGCCGCCGACCCCTCACATTGTCTACCTGTAC
AATGCGGCCATCAGCTGGCTATGCCAACAGCTGCCTCAATCCCTTGTG
TACATAGTACTCTGTGAGACCTTCGAAAACGCTTGGTGTGCGGTGAA
35 GCCCGCGGCCAGGGCAGCTCGCACGGTCAGCAATGCTCAGACAGCTG

ACGAGGAGAGGACAGAAAGCAAAGGCACCgtcgacggtaccgcggccggatccatcg
ccaccATGGT GAGCAAGGGCGAGGAGCTGTCACCGGGTGGTGCCCATCC
TGGTCGAGCTGGACGGCGACGTAAACGCCACAAGTTAGCGTGTCCGGC
GAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCTGAAGTTCATCTG
5 C'ACCA CGGCAAGCTGCCGTGCCCTGCCACCCCTCGTGACCA CCTGA
CCTACGGCGTGCAGTGCTCAGCCGCTACCCCGACCACATGAAGCAGCAC
GACTCTTCAAGTCCGCATGCCGAAGGCTACGTCCAGGAGCGCACCAC
CTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCAGGTGAAGTCG
AGGGCGACACCCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAG
10 GAGGACGGCAACATCCTGGGCACAAGCTGGAGTACAACACTACAACAGCC
ACAACGTCTATATCATGGCCACAAGCAGAAGAACGGCATCAAGGTGAA
CTTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTGCCGACC
ACTACCAGCAGAACACCCCCATCGGCACGGCCCGTGTGCTGCCGAC
AACCAACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCAACGAGAA
15 GCGCGATCACATGGTCTGCTGGAGTCGTGACCGCCGCCGGATCACTC
TCGGCATGGACGAGCTGTACAAGTAA

SEQ. ID. NO. 27: Mouse MCH1R/EGFP direct fusion

Nucleic acid sequence start codon and start and stop codons for mouse MCH1R and
20 EGFP, respectively, as well as intron borders, are highlighted:
ATGGATCTGCAAGCCTCGITGCTGTCCACTGGCCCCAATGCCAGCAACAT
CTCCGATGCCAGGATAATTACATTGGCGGGTGAAGTCAGTTGGAGTC
CTCCCTCCTCCGGATGGGTGGAAAATGGGAAGGTTTACCTCCCAAG
CCAAACTGCCTGGAAACTTATCTTACAGTTGGTGATAAGATCTGCA
25 GTCGGCTTGCCTGAAGAGGAAGAGGAGAGGAGGGGACACCAGCTAGGA
CAGAAGGGGCAGGGAGGAATAGAGATGGGGCAGAGGCACATTAGAAAC
AACAAAGGGTTGGTGACAAGACGTGAGGCAGGCTTGAGGGAAAGCTTGC
TGATGAGTCCAAATATGCTTGCAGGGGGGGGGGGGGAAATCAAGG
CTGGAGAAGCAAGCAAGCAAGACAGCAAGACAGCGGGCGGGTAGTATGT
30 GGGAGCCAGCAGAACGCGCTTGATTACCGCTATCCTGGCTCAATCCTC
TGGCCTCGCACTGGGAAATGGGTCTGAGTGGCCTGCTGTCTTCTGGC
AAAGGCTGCTGGAGCAAAAGACTTCACAGGGCGTGAGAGGATTAACCTT
TCTGGTGAATTAAGCTTCTGACATTGCAGAACGTCAATGCCTTAAATT
CTAGCTCTGAAGGAGAAGGGAAATGAAGGGAAAGAGGGAAAGAGGTGGTGT
35 GGAGAAATTCCAAGCTTCTGGGTGTAACACAGCTCCAGTCCCTACCCT

ATTGGGAAAGCCCAGACTCAGGAGACATGGTCCAAGGAAATCCCTGACA
GAAAACCGGGAGAGGGCAGGGCTGTGGAGCCTGAAACACACCCCCACACC
CATGGTACAGTCACITCTCACATATGCCTAGGAACCTATCTGAAACCTT
GCCATCTCTCTGAAAAGATGAGGCTGCAAATACACACACACACACAC
5 AC
TGTCTTCAAGCCTTTGACAAGGTTCTGGTGGATCCGGGGATATGA
AGTTGTTCTCAGCAGATATCTGGAGTCITGACTCCTGGCCCTCTGAGTAA
ATGGATGAAGCGAAGAAGAATGGGTCCTCTGAGTAACAGGTGGATCTA
GAAAATCCTATAGGAGTCACCAGGGCACGGTGGAGGAGGGTAAGGTACA
10 GAACTAACAAATAGCCCAGAGAAGGGAAACAGCAGGAGATGATTCCAGAG
ACGTAGTGACCCCAAGCTGCAAGGGAAAGCATGAGGGGCCAGCAGGAAG
GCCGACATGGCAGGTGTCAGCTCTAGATCGGAAGGCGGGTACACTTG
CTCTTCTATCCTCAGGCCACCTCCTGCACAAGGAGTGTCTCCTACATC
AACATCATCATGCCTCAGTGTGGTACCATCTGTCTCCTGGCATTGTG
15 GGAAACTCCACAGTCATTTGCCGTGGTGAAGAAATCCAAGCTGCACTG
GTGCAGCAACGTCCCTGACATCTCATCATCACACCTCTGTGGTGGATCT
GCTTTCTGCTGGCATGCCCTCATGATCCACCAAGCTCATGGTAATGG
TGTCTGGCACTTGGGAAACCATGTGCACCCTCATCACAGCCATGGACG
CCAACAGTCAGTCACCAGCACCTACATCCTGACTGCTATGCCATTGACC
20 GCTACTTGGCCACCGTCCATCCCATCTCCTCCACCAAGTCCGGAAGCCCT
CCATGGCCACCCCTGGTATGCCCTGTGGCTCTCGTTCACTAGCA
TCACTCCTGTGTGGCTCATGCCAGGTTATCCCTCCAGGGGGTGTG
TGGCTGTGGCATCCGCTACCAAACCCAGATACTGATCTTACTGGTCA
CTCTGTATCAGTTTCTGGCCTCGCCCTCCGGTTGTGGTCACTACTGC
25 TCGTACGTGAAAATACTACAGCGCATGACGTCTCGGTGGCCCCAGCCT
CTCAACGCAGCATCCGGCTTCGGACAAAGAGGGTGACCCGCACAGCCATT
GCCATCTGTCTGGCTCTCTGTGTGCTGGCGCCCTACTACGTGCTGCAG
CTGACCCAGTTGTCCATCAGCCGCCACCTCACATTGTCTACCTGTAC
AATGCGGCCATCAGCTGGTATGCCAACAGCTGCCTCAATCCCTTG
30 TACATAGTACTCTGTGAGACCTTCTGAAAACGCTTGGTGTGCGGTGAA
GCCCGCGGCCCAGGGCAGCTCGCACGGTCAGCAATGCTCAGACAGCTG
ACGAGGAGAGGACAGAAAGCAAAGGCACCATGGTGAGCAAGGGCGAGG
AGCTGTTACCGGGGTGGTGCCTCGAGCTGGACGGCGACGTA
AACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCT
35 ACGGCAAGCTGACCCCTGAAGTTCATCTGCACCAACGGCAAGCTGCCGTG

CCCTGGCCCACCCCTCGTGACCACCCGTACCTACGGCGTGCAGTGCTTCAG
CCGCTACCCCGACCACATGAAGCAGCAGCACTTCAAGTCCGCCATGC
CCGAAGGCTACGTCCAGGAGCGCACCATCTTCAAGGACGACGGCAAC
TACAAGACCCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCCTGGTGAACC
5 GCATCGAGCTGAAGGGCATCGACTTCAAGGAGGGACGGCAACATCCTGGG
GCACAAGCTGGAGTACAACACTACAACAGCCACAACGTCTATATCATGGCCG
ACAAGCAGAAGAACGGCATCAAGGTGAACCTCAAGATCCGCCACAACAT
CGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCC
ATCGGCACGGCCCCGTGCTGCTGCCGACAACCAACTACCTGAGCACCCA
10 GTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGCCTGC
TGGAGTTCGTGACCGCCGCCGGGATCACTCTGGCATGGACGAGCTGTAC
AAGTAA

SEQ. ID. NO. 28: Human short form/mouse species chimeric MCH1R-linker-

15 EGFP

Nucleic acid sequence start codon and start and stop codons for mouse MCH1R and EGFP, respectively, are highlighted and a 12 amino acid linker sequence is denoted in lower case:

ATGGACCTGGAAGCCTCGCTGCTGCCACTGGTCCCAATGCCAGCAACAC
20 CTCTGATGGCCCCGATAACCTCACTTCGGCAGGATCACCTCCTCGCACGG
GGAGCATCTCCTACATCAACATCATCATGCCTTCGGTGTTCGGCACCATCT
GCCTCCTGGCATCATCGGGAACTCCACGGTCATCTCGCGGTGCGTGAAG
AAGTCCAAGCTGCACTGGTGAACAACGTCCCCGACATCTTCATCATCAA
CCTCTCGGTAGTAGATCTCCTCTTCTCCTGGCATGCCCTCATGATCCA
25 CCAGCTCATGGCAATGGGGTGTGGCACTTGGGAGACCATGTGCACCC
TCATCACGGCCATGGATGCCAATAGTCAGTTACCCAGCACCTACATCCTG
ACCGCCATGCCATTGACCGCTACCTGCCACTGTCCACCCATCTCTTG
ACGAAGTTCCGGAAGCCCTCCATGCCACCTGGTGTGCCTTCCTGTG
GGCTCTCTCGTTCAATTAGCATCACTCCTGTGTGGCTATGCCAGGGTTAT
30 CCCCTCCAGGGGGTGTGGCACTTGGGCTGTGGCATCCGCCTACCAAACCCAG
ATACTGATCTTACTGGTCACTCTGTATCAGTTTCTGGCCTTCGCCCT
TCCGTTTGTGGTCATCACTGCTGCGTACGTGAAAATACTACAGCGCATGAC
GTCTCGGTGGCCCCAGCCTCTCAACGCAGCATCCGGCTCGGACAAAGA
GGGTGACCCGCACGCCATTGCCATCTGTCTGGCTTCTTGTGTGCTGGG
35 CGCCCTACTACGTGCTGCAGCTGACCCAGTTGTCCATCAGCCGCCGACC

CTCACATTCTACCGTACAATGCCGCATCAGCTGGCTATGCCAAC
AGCTGCCTCAATCCCTTGTCAGCATAGTACTCTGTGAGACCTTCGAAAA
CGCTTGGTGGCTGTCGGTAAGCCCGCCAGGGCAGCTCGCACGGT
CAGCAATGCTCAGACAGCTGACGAGGAGAGGACAGAAAGCAAAGGCACC
5 gtcgacggtaccggccggccggatccatgcacc ATGGTGAGCAAGGGCGAGGAGCTGTT
CACCGGGGTGGTGCCCATCCTGGTCAGCTGGACGGCACGTAAACGGCC
ACAAGTTCAAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAA
GCTGACCTGAAGTTCATCTGCACCACCGCAAGCTGCCGTGCCCTGGC
CCACCCCTCGTGACCAACCTGACCTACGGCGTGCAGTGCITCAGCCGCTAC
10 CCCGACCATGAAGCAGCACGACTTCAAGTCCGCATGCCGAAGG
CTACGTCCAGGAGCGCACCATCTTCAAGGACGACGGCAACTACAAGA
CCCGCGCCGAGGTGAAGTTGAGGGCGACACCCCTGGTAACCGCATCGAG
CTGAAGGGCATCGACTTCAAGGAGGACGGAACATCCTGGGCACAAGC
TGGAGTACAACATACAACAGCCACAACGTCTATATCATGGCCACAAGCAG
15 AAGAACGGCATCAAGGTGAACCTCAAGATCCGCCACAACATCGAGGACG
GCAGCGTGCAGCTGCCGACCACTACCGAGCAGAACACCCCCATCGCGAC
GGCCCCGTGCTGCCGACAACCAACTACCTGAGCACCCAGTCCGCCCT
GAGCAAAGACCCAACGAGAAGCGCGATCACATGGTCCCTGCTGGAGTTG
TGACCGCCGCCGGATCACTCTCGGCATGGACGAGCTGTACAAGTAA
20

SEQ. ID. NO. 29: Human long form/mouse species chimeric MCH1R-linker-EGFP

Nucleic acid sequence start codon and start and stop codons for mouse MCH1R and EGFP, respectively, are highlighted and a 12 amino acid linker sequence is denoted in lower case:

ATGTCAGTGGGAGCCATGAAGAAGGGAGTGGGGAGGGCAGTTGGCTTG
GAGGCAGCGCTGCCAGGCTACGGAGGAAGACCCCTCCCAACTGC
GGGGCTTGCCTCCGGACAAGGTGGCAGGCCCTGGAGGGCTGCCGCAGC
CTCGTGGGTGGAGGGAGCTCAGCTGGTTGTGGAGCAGGCGACCGG
30 CACTGGCTGGATGGACCTGGAAGCCTCGCTGCCACTGGTCCCAACG
CCAGCAACACCTCTGATGGCCCCGATAACCTCACTTCGGCAGGATCACCT
CCTCGCACGGGAGCATCTCCTACATCAACATCATGCCITCGGTGTT
GGCACCATCTGCCCTGGCATCGGGAACTCCACGGTCACTTCGCG
GTCGTGAAGAAGTCCAAGCTGCACTGGTCAACAACGTCCCCGACATCTT
35 CATCATCAACCTCTCGGTAGTAGATCTCCTCTTCTCCTGGGCATGCCCTT

CATGATCCACCAGCTCATGGCAATGGGTGTGGCACTTGGGAGACCA
TGTGCACCTCATCACGCCATGGATGCCAATAGTCAGTCACCAGCACC
TACATCCTGACCGCCATGCCATTGACCGCTACCTGCCACTGTCCACCC
ATCTCTCCACGAAGTCCGGAAGCCCTCATGCCACCCGGTATCTGC
5 CTCCTGTGGCTCTCGTTAGCATCACTCCTGTGTGGCTATGCC
AGGCTTATCCCCCTCCAGGGGGCTGTGGCTGTGGCATCCGCCTACCA
AACCCAGATACTGATCTTACTGGTCACTCTGTATCAGTTTCTGGCCT
TCGCCCTCCGTTGTGGTCACTGCTGCGTACGTAAAATACTACAGC
GCATGACGTCTCGGTGGCCCCAGCCTCTAACGCAGCAGCATCCGGCTCGG
10 ACAAAAGAGGGTACCCGCACAGCATTGCCATCTGTCTGGCTTCTTG
TGCTGGCGCCCTACTACGTGCTGCAGCTGACCCAGTTGTCCATCAGCCGC
CCGACCCCTCACATTGCTACCTGTACAATGCCGCATCAGCTGGCTAT
GCCAACAGCTGCCCTCAATCCCTTGTACATAGTACTCTGTGAGACCTT
CGAAAACGCTTGGTGTGCGGTGAAGGCCGCCAGGGCAGCTCG
15 CACGGTCAGCAATGTCAGACAGCTGACGAGGAGAGGACAGAAAGCAA
GGCACCGtcgacggtaccggggccggatccatgccaccATGGTGAGCAAGGGCGAGGA
GCTGTTACCGGGGTGGTGCCCCTCCTGGTCGAGCTGGACGGCACGTAA
ACGCCACAAGTTAGCGTGTCCGGAGGGCGAGGGCGATGCCACCTAC
GGCAAGCTGACCCCTGAAGITCATCTGCAACCACGGCAAGCTGCCGTGCC
20 CTGGCCCACCTCGTGAACCAACCTGACCTACGGCGTGCAGTGCTTCAGCC
GCTACCCCGACCACATGAAGCAGCACGACTTCTCAAGTCCGCATGCC
GAAGGCTACGTCCAGGAGCGCACCATCTTCTCAAGGACGACGGCAACTA
CAAGACCCGCCGAGGTGAAGTTGAGGGGACACCCCTGGTAACCGC
ATCGAGCTGAAGGGCATCGACTCAAGGAGGACGGCAACATCCTGGGC
25 ACAAGCTGGAGTACAACACAGCCACAACGTCTATATCATGGCCGAC
AAGCAGAAGAACGGCATCAAGGTGAACCTCAAGATCCGCCACAACATCG
AGGACGGCAGCGTGCAGCTGCCGACCACTACCAAGCAGAACACCCCCATC
GGCGACGGCCCCGTGCTGCTGCCGACAACCACTACCTGAGCACCCAGTC
CGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCTGCTGG
30 AGTCGTGACCGCCGCCGGATCACTCTCGGCATGGACGAGCTGTACAAG
TAA

Example 2: Generation of Chimeric and Fusion Proteins

DNA vectors encoding fusion proteins between a MCH-R receptor
35 (MCH1R) and several different superbright variants of Green Fluorescent Protein

(GFP) were generated. GFP variants were fused either via a 12 amino acid linker: TCGACGGTACCGCGGGCCCGGGATCCATGCCACC (SEQ. ID. NO. 30), amino acid sequence: VDGTAGPGSIAT (SEQ. ID. NO. 31) (linker fusions) or directly to the C-terminus of MCH1R (direct fusions).

5

Mouse MCH1R-linker-GFP Variant Fusion Constructs

Initially, mouse MCH1R was fused in frame via the linker to Enhanced Green Fluorescent Protein (EGFP). MCH1R was PCR-amplified (95°C for 5 minutes; 95°C for 30 seconds, 60°C for 45 seconds, 68°C for 3.5 minutes, for 15 cycles; 68°C for 7 minutes) from a full-length mouse MCH1R genomic DNA lambda clone utilizing a high fidelity polymerase mix (Expand High Fidelity PCR System from Boehringer Mannheim) and PCR primers [MCH1R (Eco RI) 5': GCGAATTCAACCATGGATCTGCAAGCCTCG (SEQ. ID. NO. 32), MCH1R (Sal I) 3': GCGTCGACGGTGCCCTTGCTTTCTGTCC (SEQ. ID. NO. 33)] that generated Eco RI and Sal I enzymatic restriction sites at the N- and C-terminus, respectively. The MCH1R N-terminal PCR primer was also designed to introduce a Kozak consensus sequence for translation which contained an Nco I site (5'-ACCATGG-3'), and the MCH1R C-terminal PCR primer was also designed to eliminate the endogenous stop codon present in the mouse MCH1R gene. The resulting PCR product was phenol/chloroform extracted, restriction digested with Eco RI and Sal I, gel purified, and subcloned in frame into the multicloning site of Clontech's pEGFP-N3 vector between Eco RI and Sal I sites. Several resulting clones for this construct were sequenced to identify a clone with an entirely correct nucleotide sequence. This clone was named mMCH1R-l-EGFP for mouse MCH1R-linker-EGFP.

An approximately 760 bp Sal I to Not I fragment of mMCH1R-l-EGFP was excised, gel purified, and subcloned into the multicloning site of pBluescript (SK+) (Stratagene) between Sal I and Not I sites. An approximately 710 bp Nco I to Bsr G1 fragment of EGFP was excised from the resulting pBluescript-EGFP vector and replaced with the corresponding Nco I to Bsr G1 fragment of either Emerald, Topaz, or W1B (other superbright GFP variants), which were excised from vectors pRSET-Emerald, pRSET-Topaz, and pRSET-W1B, respectively. pRSET-Emerald, pRSET-Topaz, and pRSET-W1B were obtained from Aurora Biosciences Co. Sal I to Not I fragments containing either Emerald, Topaz, or W1B were excised from the resulting pBluescript-Emerald, pBluescript-Topaz, and pBluescript-W1B vectors, respectively. Appropriate fragments were gel purified and subcloned into mMCH1R-

1-EGFP digested with Sal I and Not I, replacing the Sal I to Not I EGFP fragment with the corresponding Sal I to Not I fragment from either Emerald, Topaz, or W1B. Several clones for each construct were sequenced to confirm the presence of the appropriate GFP variant. The resulting vectors were named mMCH1R-1-Emerald,
5 mMCH1R-1-Topaz, and mMCH1R-1-W1B for mouse MCH1R-linker-Emerald, mouse MCH1R-linker-Topaz, and mouse MCH1R-linker-W1B, respectively.

Mouse MCH1R/GFP Variant Direct Fusion Constructs

A two step PCR strategy was employed to generate the direct fusion constructs. First, mouse MCH1R, EGFP, and Emerald were PCR-amplified from a full-length mouse MCH1R genomic DNA lambda clone, Clontech's pEGFP-N3 vector, and Aurora's pRSET-Emerald vector, respectively. Mouse MCH1R was PCR-amplified according to the previously mentioned conditions utilizing the same N-terminal PCR primer [MCH1R (Eco RI) 5': GCGAATTCAACATGGATCTGCA
10 AGCCTCG (SEQ. ID. NO. 32)], but in this case a different C-terminal PCR primer was employed. The C-terminal PCR primer [MCH1R (EGFP/Emerald) 3': CCTTGCTCACCATGGTGCCTTGCTTCTGTCC (SEQ. ID. NO. 34)] eliminated the endogenous stop codon of mouse MCH1R as before and introduced a region of nucleotide sequence complementary to the nucleotide sequence of the N-terminus of
15 EGFP.
20

EGFP and Emerald were PCR-amplified (95°C for 5 minutes; 95°C for 30 seconds, 60°C for 45 seconds, 68°C for 1.5 minutes, for 15 cycles; 68°C for 7 minutes) separately with a high fidelity polymerase mix (Advantage HF-2 from Clontech) from their respective templates utilizing a common N-terminal PCR primer [EGFP/Emerald (MCH1R) 5': CAGAAAGCAAAGGCACCATGGTGAGCAA
25 GGGCGAGGAGC (SEQ. ID. NO. 35)] that generated a region of nucleotide sequence complementary to the C-terminus of mouse MCH1R and C-terminal PCR primers [EGFP 3': GGCAGATCCTCTAGAGTCGCGGCC (SEQ. ID. NO. 36), or Emerald (EGFP) 3': GCTCTAGAGTCGCGGCCGCTTACTTGTACAGCTCGTCC
30 (SEQ. ID. NO. 37)] that generated a Not I site at the C-terminus. The resulting PCR products were electrophoresed on an agarose gel and the appropriate fragments were gel purified.

In a second PCR step, PCR reactions were set up between the previously generated mouse MCH1R and EGFP, or mouse MCH1R and Emerald
35 PCR products. Following an initial 5 minute denaturation step at 95°C, two rounds of

thermocycling (95°C for 30 seconds, 60°C for 45 seconds, 68°C for 4 minutes) were performed in the absence of PCR primers. This allowed the mouse MCH1R and GFP variants to anneal at their complementary regions and to be filled in by the high fidelity polymerase mix (Expand High Fidelity PCR System from Boehringer

5 Mannheim), yielding double stranded template DNA.

Subsequently, the common N-terminal mouse MCH1R [MCH1R (Eco RI) 5': GCGAATTCAACCATGGATCTGCAAGCCTCG (SEQ. ID. NO. 32)] and appropriate C-terminal PCR primers [EGFP 3': GGCGGATCCTCTAGAGTC GCGGCC (SEQ. ID. NO. 36) or Emerald (EGFP) 3': GCTCTAGAGTCGCGG

10 CCGCTTACTTGTACAGCTCGTCC (SEQ. ID. NO. 37)] were added to the reactions and thermocycling was continued for an additional fifteen cycles followed by a final extension at 68°C for 7 minutes. The resulting PCR products were phenol/chloroform extracted, restriction digested with Eco RI and Not I, electrophoresed on an agarose gel, and appropriate fragments were gel purified.

15 These Eco RI to Not I fragments represent direct fusions between either mouse MCH1R and EGFP, or mouse MCH1R and Emerald. Clontech's pEGFP-N3 vector was restriction digested with Eco RI and Not I liberating an approximately 780 bp Eco RI to Not I EGFP fragment. This restriction digest was electrophoresed on an agarose gel and the approximately 3.9 Kb pEGFP-N3 vector backbone was gel purified. Eco RI to Not I mouse MCH1R/EGFP or mouse MCH1R/Emerald direct fusion fragments were subcloned into the pEGFP-N3 vector backbone between Eco RI and Not I sites. Several resulting clones for each of these two constructs were sequenced to identify clones with correct nucleotide sequence; however, no clones with entirely correct nucleotide sequences were identified.

20 25 Fortunately, several clones for each of the two constructs only had nucleotide mismatches in the intron region of mouse MCH1R, and therefore, were not expected to effect the functionality of the resulting fusion proteins. These clones were named mMCH1R/EGFP and mMCH1R/Emerald for mouse MCH1R/EGFP direct fusion and mouse MCH1R/Emerald direct fusion, respectively.

30

Human Short and Long Form/Mouse Species Chimeric
MCH1R-linker-GFP Variant Fusion Constructs

The initial mouse MCH1R-linker-GFP variant fusion constructs were modified to generate both human short form and human long form/mouse species

chimeric MCH1R-linker-GFP variant fusion constructs. An approximately 1.7 kb Hind III to Bsp EI fragment of the mouse MCH1R gene containing exon 1, the intron, and 127 amino acids of exon 2 was excised from the various mouse MCH1R-linker-GFP variant fusion constructs and replaced by either an approximately 470 bp Hind III to Bsp EI fragment from the wild-type human MCH1R short form or an approximately 670 bp Hind III to Bsp EI fragment from the wild-type human MCH1R long form.

Several clones for each construct were sequenced to confirm the presence of the N-terminal region of either the human MCH1R short or long forms. 10 These clones were named hshort/mMCH1R-l-GFP variant or hlong/mMCH1R-l-GFP variant for human short form/mouse species chimeric MCH1R-linker-GFP variant and human long form/mouse species chimeric MCH1R-linker-GFP variant, respectively.

15 Example 3: Functional Evaluation of MCH1R/GFP Variant Fusion Proteins

Both HEK293 Aequorin (National Institutes of Health) and CHO mammalian cell lines were transiently transfected with the various MCH1R/GFP variant fusion constructs, as well as the appropriate control constructs. Transfection was performed using Lipofectamine 2000 (Gibco BRL) per the manufacturer 20 recommended protocol. Approximately 48 hours after transfection cells were harvested, stimulated with various concentrations of human MCH, and assayed for either aequorin bioluminescence (HEK293 Aequorin cells) or cAMP production (CHO cells). Aequorin bioluminescence is a representative measure of intracellular Ca²⁺ mobilization. cAMP production was measured with the Adenylyl Cyclase 25 Activation FlashPlate Assay (NEN Life Science Products, Inc.).

Following transient transfection of the mMCH1R-linker-EGFP construct (MCH-R-l-EGFP) into HEK293 Aequorin cells, the resulting fusion protein exhibited functional activity comparable to that of the wild-type human MCH1R short form (MCH-R wt). By this functional assay, the EC₅₀ value for mMCH1R-l-EGFP 30 was nearly identical to that of the wild-type human short form receptor (Figure 1).

Following transient transfections of the mMCH1R-l-EGFP and mMCH1R/EGFP fusion constructs into CHO cells, the resulting fusion proteins exhibited functional activity comparable to that of the wild-type human MCH1R short form. By this functional assay, the EC₅₀ values for mMCH1R-l-EGFP and 35 mMCH1R/EGFP were comparable to that of the wild-type human receptor (Table 1).

Transient transfections with the corresponding Emerald constructs yielded similar results (data not shown).

Table 1

5

Receptor	EC50 (nM)
Wild-type Human MCH1R Short Form	2.166
Mouse MCH1R/EGFP	0.819
Mouse MCH1R-l-EGFP	3.199

Following transient transfections of the human short form/mouse species chimeric MCH1R-l-EGFP (HuShort/mMCH1R-l-EGFP) and human long form/mouse species chimeric MCH1R-l-EGFP (HuLong/mMCH1R-l-EGFP) 10 constructs into HEK293 cells, the resulting fusion proteins exhibited functional activity comparable to that of the wild-type human MHC1R short and long forms, respectively. By this functional assay, the EC50 value for each fusion proteins was nearly identical to that of the corresponding wild-type human receptor (Table 2).

15

Table 2

Receptor	EC50 (nM)
Wild-type Human MCH1R Short Form	22.27
HuShort/mMCH1R-l-EGFP	19.54
Wild-type Human MCH1R Long Form	196.7
HuLong/mMCH1R-l-EGFP Form	217.5

Following transient transfections of the human short form/mouse species chimeric MCH1R-l-EGFP (HuShort/mMCH1R-l-EGFP) and human long form/mouse species chimeric MCH1R-l-EGFP (HuLong/mMCH1R-l-EGFP) 20 constructs into CHO cells, the resulting fusion proteins exhibited functional activity comparable to or less than that of the wild-type human MHC1R short and long forms, respectively (Table 3). By this functional assay, the EC50 value for the human short form/mouse species chimeric MCH1R-l-EGFP fusion protein was comparable to that 25 of the corresponding wild-type human receptor, whereas, the human long form/mouse

species chimeric MCH1R-I-EGFP fusion protein had an EC₅₀ value approximately 7.5-fold higher than that of its corresponding wild-type control.

Table 3

5

Receptor	EC ₅₀ (nM)
Wild-type Human MCH1R Short Form	1.029
Wild-type Human MCH1R Long Form	1.515
HuShort/mMCH1R-I-EGFP	1.565
HuLong/mMCH1R-I-EGFP	11.580

Transient expression of all the MCH1R/GFP variant fusion proteins that underwent functional evaluation resulted in fluorescence primarily associated with the plasma membrane in both HEK293 and CHO cells (data not shown). This 10 pattern of fluorescence is consistent with a predominant membrane associated localization.

Example 4: Generation of Stable Cell Lines

Wild-type CHO cells were transfected using SuperFect (Qiagen) and 15 either mouse MCH-1R-EGFP or human short/mouse species chimeric MCH-1R-EGFP. Forty-eight hours after transfection, transfected cells were subjected to positive selection for approximately ten days in media containing G418. Following selection, MCH-1R-EGFP expressing CHO cells were bulk sorted by Fluorescence Assisted Cell Sorting (FACS) for one or two rounds on the basis of fluorescence 20 intensity to increase the population of cells expressing EGFP. Following bulk sorts, individual clones of varying fluorescence intensities were isolated by FACS and expanded.

Fluorometric Microvolume Assay Technology (FMAT) was initially employed to screen a large number of stable clones by whole cell binding with a 25 fluoresceinyl labeled MCH derivative (SymJz-MCH, PE Biosystems) to identify those clones with good specific binding windows. Several clones exhibiting specific binding windows greater than 3-fold were further evaluated for MCH binding with the SPA-based Binding Assay. Cells from individual clones were dissociated in enzyme free dissociation media and cell membranes were prepared and subsequently tested for

their ability to bind [¹²⁵I]Phe¹³Tyr¹⁹-MCH in the presence of human MCH. CHO cell lines expressing either mouse MCH-1R-EGFP or human short/mouse species chimeric MCH-1R-EGFP (Figure 4) displayed IC50 values with MCH that were indistinguishable from the corresponding IC50 values obtained with a CHO cell line expressing the wild-type human short isoform of MCH-1R.

The functional activity of these clones was evaluated with the cAMP Flashplate Assay (Figures 2 and 3). CHO cell lines expressing either mouse MCH-1R-EGFP (Figure 2) or human short/mouse species chimeric MCH-1R-EGFP (Figure 3) displayed EC50 values with human MCH that were indistinguishable from the EC50 value obtained with a CHO cell line expressing the wild-type human short isoform of MCH-1R.

The subcellular localization of the MCH-1R-EGFP fusion proteins were determined by confocal microscopy utilizing EGFP fluorescence as a marker for MCH-1R expression. CHO cell lines stably expressing either mouse MCH-1R-EGFP or human short/mouse species chimeric MCH-1R-EGFP displayed EGFP fluorescence primarily associated with the plasma membrane, demonstrating that these MCH-1R-EGFP fusion proteins are primarily associated with the plasma membrane.

Other embodiments are within the following claims. While several embodiments have been shown and described, various modifications may be made without departing from the spirit and scope of the present invention.

WHAT IS CLAIMED IS:

1. A fusion protein comprising:
 - a) a melanin concentrating hormone receptor polypeptide region comprising a sequence selected from the group consisting of: SEQ. ID. NO. 1, SEQ. ID. NO. 2, SEQ. ID. NO. 3, SEQ. ID. NO. 4, and SEQ. ID. NO. 5; and
 - b) a fluorescent polypeptide region joined directly, or through a linker, to the carboxy side of said melanin concentrating hormone receptor polypeptide region.
- 10 2. The protein of claim 1, wherein said fluorescent polypeptide region consists of an amino acid sequence selected from the group consisting of SEQ. ID. NO. 6, SEQ. ID. NO. 7, SEQ. ID. NO. 8, SEQ. ID. NO. 9, and SEQ. ID. NO. 10.
- 15 3. The protein of claim 2, wherein said melanin concentrating hormone polypeptide region consists of a sequence selected from the group consisting of: SEQ. ID. NO. 1, SEQ. ID. NO. 2, SEQ. ID. NO. 3, SEQ. ID. NO. 4, and SEQ. ID. NO. 5.
- 20 4. The protein of claim 3, wherein said protein consists essentially of said melanin concentrating hormone receptor polypeptide region and said fluorescent polypeptide region.
- 25 5. The protein of claim 4, wherein said protein consists of the amino acid sequence of SEQ. ID. NO. 11 or SEQ. ID. NO. 12.
- 30 6. The protein of claim 1, wherein said melanin concentrating hormone polypeptide region is a chimeric polypeptide comprising (a) an MCH binding region from a first species and (b) a transmembrane and intracellular domain region from a second species joined directly, or though a linker, to the carboxy side of said MCH binding region.
- 35 7. The protein of claim 6, wherein said fluorescent polypeptide region consists of an amino acid sequence selected from the group consisting of: SEQ. ID. NO. 6, SEQ. ID. NO. 7, SEQ. ID. NO. 8, SEQ. ID. NO. 9, and SEQ. ID. NO. 10.

8. The protein of claim 7, wherein said protein consists of the amino acid sequence of SEQ. ID. NO. 13 or SEQ. ID. NO. 14.

5 9. A chimeric melanin concentrating hormone protein comprising:
 a) a melanin concentrating hormone binding region
characteristic of a human melanin concentrating hormone receptor;
 b) a transmembrane domain characteristic of a non-human
melanin concentrating hormone receptor; and
10 c) an intracellular domain characteristic of a non-human
melanin concentrating hormone receptor.

10. The protein of claim 9, wherein said protein comprises a melanin concentrating hormone receptor polypeptide having a sequence similarity of at least 75% with either SEQ. ID. NO. 4 or SEQ. ID. NO. 5.
15

11. The protein of claim 10, wherein said protein comprises the sequence of SEQ. ID. NO. 4 or SEQ. ID. NO. 5.

20 12. The protein of claim 11, wherein said protein consists of the sequence of SEQ. ID. NO. 4 or SEQ. ID. NO. 5.

13. A nucleic acid comprising a nucleotide sequence encoding for the protein of claim 1.
25

14. The nucleic acid of claim 13, wherein said nucleotide sequence is a contiguous sequence.

15. The nucleic acid of claim 13, wherein said nucleotide sequence
30 is selected from the group consisting of SEQ. ID. NO. 26, SEQ. ID. NO. 27, SEQ. ID. NO. 28 and SEQ. ID. NO. 29.

16. A nucleic acid comprising a nucleotide sequence encoding for the protein of claim 9.
35

17. The nucleic acid of claim 16, wherein said nucleotide sequence
is a contiguous sequence.

18. The nucleic acid of claim 16, wherein said nucleotide sequence
5 is selected from the group consisting of SEQ. ID. NO. 19 and SEQ. ID. NO. 20.

19. An expression vector comprising the nucleic acid of claim 13.

20. An expression vector comprising the nucleic acid of claim 16.

10 21. A recombinant cell comprising the nucleic acid of claim 13.

22. The recombinant cell of claim 21, wherein said nucleic acid is
present in an expression vector.

15 23. The recombinant cell of claim 21, wherein said nucleic acid is
present in the genome of said cell.

20 24. A recombinant cell comprising the nucleic acid of claim 16.

25. The recombinant cell of claim 24, wherein said nucleic acid is
present in an expression vector.

26. The recombinant cell of claim 24, wherein said nucleic acid is
25 present in the genome of said cell.

27. A non-human transgenic animal comprising the nucleic acid of
claim 13.

30 28. A non-human transgenic animal comprising the nucleic acid of
claim 16.

29. A method for assaying for melanin concentrating hormone
receptor active compounds comprising the steps of:

- a) contacting the cell of claim 21 with a test preparation comprising one or more test compounds; and
- b) measuring the effect of said test preparation on one or more melanin concentrating hormone receptor activities.

5

30. A method for assaying for melanin concentrating hormone receptor active compounds comprising the steps of:

- a) contacting the cell of claim 24 with a test preparation comprising one or more test compounds; and
- b) measuring the effect of said test preparation on one or more melanin concentrating hormone receptor activities.

10

1/3

mMCH1R-l-EGFP Aequorin Assay

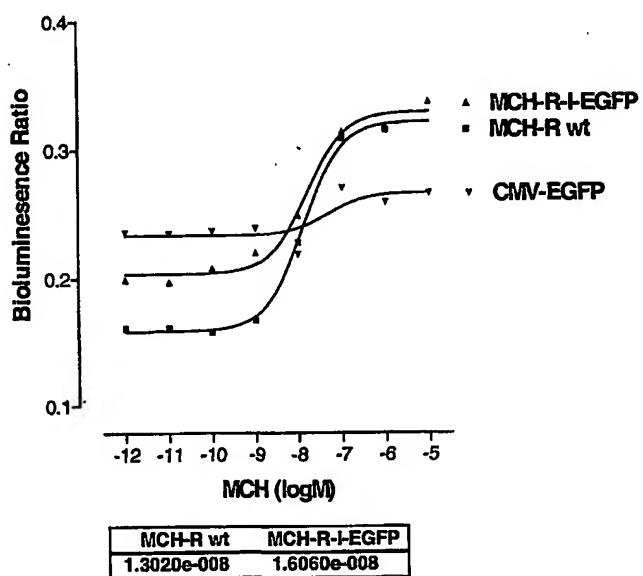


Fig. 1

2/3

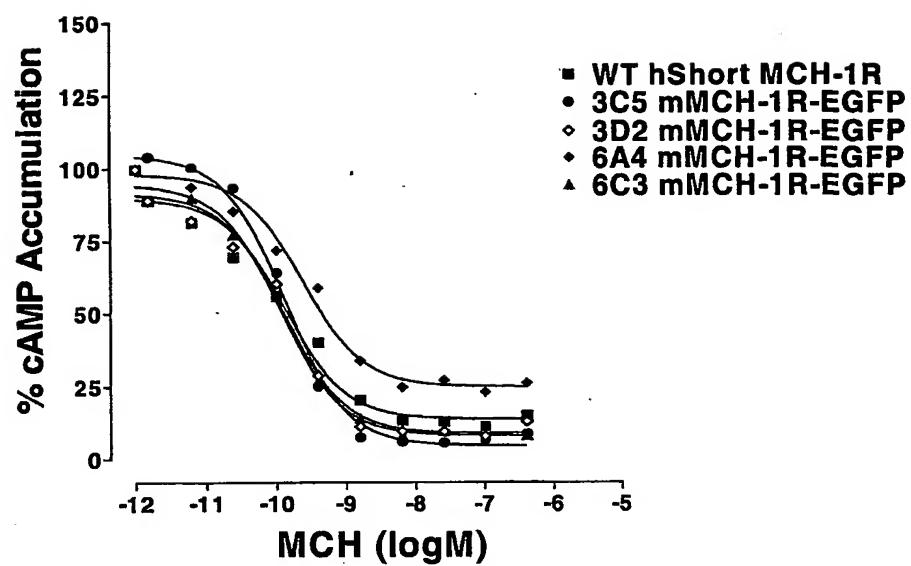


Fig. 2

3/3

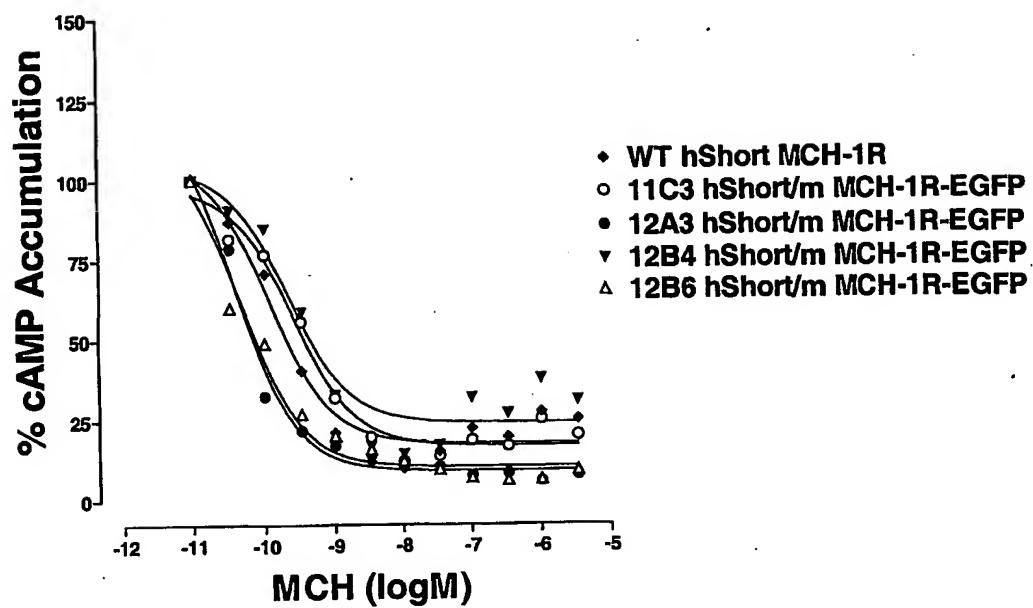


Fig. 3

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290 295 300
Cys Leu Asn Pro Phe Val Tyr Ile Val Leu Cys Glu Thr Phe Arg Lys
305 310 315 320
Arg Leu Val Leu Ser Val Lys Pro Ala Ala Gln Gly Gln Leu Arg Thr
325 330 335
Val Ser Asn Ala Gln Thr Ala Asp Glu Glu Arg Thr Glu Ser Lys Gly
340 345 350
Thr

<210> 5
<211> 422
<212> PRT
<213> Artificial Sequence

<220>

<223> Human long form/mouse species chimeric MCH1R

<400> 5

Met Ser Val Gly Ala Met Lys Lys Gly Val Gly Arg Ala Val Gly Leu
 1 5 10 15
 Gly Gly Gly Ser Gly Cys Gln Ala Thr Glu Glu Asp Pro Leu Pro Asn
 20 25 30
 Cys Gly Ala Cys Ala Pro Gly Gln Gly Gly Arg Arg Trp Arg Leu Pro
 35 40 45
 Gln Pro Ala Trp Val Glu Gly Ser Ser Ala Arg Leu Trp Glu Gln Ala
 50 55 60
 Thr Gly Thr Gly Trp Met Asp Leu Glu Ala Ser Leu Leu Pro Thr Gly
 65 70 75 80
 Pro Asn Ala Ser Asn Thr Ser Asp Gly Pro Asp Asn Leu Thr Ser Ala
 85 90 95
 Gly Ser Pro Pro Arg Thr Gly Ser Ile Ser Tyr Ile Asn Ile Ile Met
 100 105 110
 Pro Ser Val Phe Gly Thr Ile Cys Leu Leu Gly Ile Ile Gly Asn Ser
 115 120 125
 Thr Val Ile Phe Ala Val Val Lys Lys Ser Lys Leu His Trp Cys Asn
 130 135 140
 Asn Val Pro Asp Ile Phe Ile Ile Asn Leu Ser Val Val Asp Leu Leu
 145 150 155 160
 Phe Leu Leu Gly Met Pro Phe Met Ile His Gln Leu Met Gly Asn Gly
 165 170 175
 Val Trp His Phe Gly Glu Thr Met Cys Thr Leu Ile Thr Ala Met Asp
 180 185 190
 Ala Asn Ser Gln Phe Thr Ser Thr Tyr Ile Leu Thr Ala Met Ala Ile
 195 200 205
 Asp Arg Tyr Leu Ala Thr Val His Pro Ile Ser Ser Thr Lys Phe Arg
 210 215 220
 Lys Pro Ser Met Ala Thr Leu Val Ile Cys Leu Leu Trp Ala Leu Ser
 225 230 235 240
 Phe Ile Ser Ile Thr Pro Val Trp Leu Tyr Ala Arg Leu Ile Pro Phe
 245 250 255
 Pro Gly Gly Ala Val Gly Cys Gly Ile Arg Leu Pro Asn Pro Asp Thr
 260 265 270
 Asp Leu Tyr Trp Phe Thr Leu Tyr Gln Phe Phe Leu Ala Phe Ala Leu
 275 280 285
 Pro Phe Val Val Ile Thr Ala Ala Tyr Val Lys Ile Leu Gln Arg Met
 290 295 300
 Thr Ser Ser Val Ala Pro Ala Ser Gln Arg Ser Ile Arg Leu Arg Thr
 305 310 315 320
 Lys Arg Val Thr Arg Thr Ala Ile Ala Ile Cys Leu Val Phe Phe Val
 325 330 335
 Cys Trp Ala Pro Tyr Tyr Val Leu Gln Leu Thr Gln Leu Ser Ile Ser
 340 345 350
 Arg Pro Thr Leu Thr Phe Val Tyr Leu Tyr Asn Ala Ala Ile Ser Leu
 355 360 365
 Gly Tyr Ala Asn Ser Cys Leu Asn Pro Phe Val Tyr Ile Val Leu Cys
 370 375 380
 Glu Thr Phe Arg Lys Arg Leu Val Leu Ser Val Lys Pro Ala Ala Gln
 385 390 395 400
 Gly Gln Leu Arg Thr Val Ser Asn Ala Gln Thr Ala Asp Glu Glu Arg
 405 410 415
 Thr Glu Ser Lys Gly Thr
 420

<210> 6

<211> 238

<212> PRT

<213> Aequorea Victoria

<400> 6
 Met Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val
 1 5 10 15
 Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu
 20 25 30
 Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys
 35 40 45
 Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Phe
 50 55 60
 Ser Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln
 65 70 75 80
 His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg
 85 90 95
 Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val
 100 105 110
 Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile
 115 120 125
 Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn
 130 135 140
 Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly
 145 150 155 160
 Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val
 165 170 175
 Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro
 180 185 190
 Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser
 195 200 205
 Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val
 210 215 220
 Thr Ala Ala Gly Ile Thr His Gly Met Asp Glu Leu Tyr Lys
 225 230 235

<210> 7

<211> 239

<212> PRT

<213> Artificial Sequence

<220>

<223> GFP derivative

<400> 7

Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
 1 5 10 15
 Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
 20 25 30
 Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
 35 40 45
 Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
 50 55 60
 Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
 65 70 75 80
 Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
 85 90 95
 Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
 100 105 110
 Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
 115 120 125
 Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
 130 135 140
 Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
 145 150 155 160

Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
 165 170 175
 Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
 180 185 190
 Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
 195 200 205
 Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
 210 215 220
 Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
 225 230 235

<210> 8

<211> 239

<212> PRT

<213> Artificial Sequence

<220>

<223> GFP derivative

<400> 8

Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
 1 5 10 15
 Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
 20 25 30
 Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
 35 40 45
 Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
 50 55 60
 Leu Thr Tyr Gly Val Gln Cys Phe Ala Arg Tyr Pro Asp His Met Lys
 65 70 75 80
 Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
 85 90 95
 Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
 100 105 110
 Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
 115 120 125
 Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
 130 135 140
 Asn Tyr Asn Ser His Lys Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn
 145 150 155 160
 Gly Ile Lys Val Asn Phe Lys Thr Arg His Asn Ile Glu Asp Gly Ser
 165 170 175
 Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
 180 185 190
 Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
 195 200 205
 Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
 210 215 220
 Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
 225 230 235

<210> 9

<211> 239

<212> PRT

<213> Artificial Sequence

<220>

<223> GFP derivative

<400> 9

Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
 1 5 10 15

Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly
 20 25 30
 Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
 35 40 45
 Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
 50 55 60
 Phe Gly Tyr Gly Val Gln Cys Phe Ala Arg Tyr Pro Asp His Met Arg
 65 70 75 80
 Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
 85 90 95
 Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
 100 105 110
 Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
 115 120 125
 Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
 130 135 140
 Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn
 145 150 155 160
 Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
 165 170 175
 Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
 180 185 190
 Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Tyr Gln Ser Ala Leu
 195 200 205
 Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
 210 215 220
 Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
 225 230 235

<210> 10

<211> 239

<212> PRT

<213> Artificial Sequence

<220>

<223> GFP derivative

<400> 10
 Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu
 1 5 10 15
 Val Glu Leu Asp Gly Asp Val Asn Gly His Arg Phe Ser Val Ser Gly
 20 25 30
 Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile
 35 40 45
 Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr
 50 55 60
 Leu Thr Trp Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys
 65 70 75 80
 Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu
 85 90 95
 Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu
 100 105 110
 Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly
 115 120 125
 Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr
 130 135 140
 Asn Tyr Ile Ser His Asn Val Tyr Ile Thr Ala Asp Lys Gln Lys Asn
 145 150 155 160
 Gly Ile Lys Ala His Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser
 165 170 175
 Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly
 180 185 190

Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu
 195 200 205
 Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe
 210 215 220
 Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
 225 230 235

<210> 11
<211> 604
<212> PRT
<213> Artificial Sequence

<220>
<223> Mouse MCH1R-linker-EGFP

<400> 11
Met Asp Leu Gln Ala Ser Leu Leu Ser Thr Gly Pro Asn Ala Ser Asn
 1 5 10 15
Ile Ser Asp Gly Gln Asp Asn Phe Thr Leu Ala Gly Pro Pro Pro Arg
 20 25 30
Thr Arg Ser Val Ser Tyr Ile Asn Ile Ile Met Pro Ser Val Phe Gly
 35 40 45
Thr Ile Cys Leu Leu Gly Ile Val Gly Asn Ser Thr Val Ile Phe Ala
 50 55 60
Val Val Lys Lys Ser Lys Leu His Trp Cys Ser Asn Val Pro Asp Ile
 65 70 75 80
Phe Ile Ile Asn Leu Ser Val Val Asp Leu Leu Phe Leu Leu Gly Met
 85 90 95
Pro Phe Met Ile His Gln Leu Met Gly Asn Gly Val Trp His Phe Gly
 100 105 110
Glu Thr Met Cys Thr Leu Ile Thr Ala Met Asp Ala Asn Ser Gln Phe
 115 120 125
Thr Ser Thr Tyr Ile Leu Thr Ala Met Ala Ile Asp Arg Tyr Leu Ala
 130 135 140
Thr Val His Pro Ile Ser Ser Thr Lys Phe Arg Lys Pro Ser Met Ala
 145 150 155 160
Thr Leu Val Ile Cys Leu Leu Trp Ala Leu Ser Phe Ile Ser Ile Thr
 165 170 175
Pro Val Trp Leu Tyr Ala Arg Leu Ile Pro Phe Pro Gly Gly Ala Val
 180 185 190
Gly Cys Gly Ile Arg Leu Pro Asn Pro Asp Thr Asp Leu Tyr Trp Phe
 195 200 205
Thr Leu Tyr Gln Phe Phe Leu Ala Phe Ala Leu Pro Phe Val Val Ile
 210 215 220
Thr Ala Ala Tyr Val Lys Ile Leu Gln Arg Met Thr Ser Ser Val Ala
 225 230 235 240
Pro Ala Ser Gln Arg Ser Ile Arg Leu Arg Thr Lys Arg Val Thr Arg
 245 250 255
Thr Ala Ile Ala Ile Cys Leu Val Phe Phe Val Cys Trp Ala Pro Tyr
 260 265 270
Tyr Val Leu Gln Leu Thr Gln Leu Ser Ile Ser Arg Pro Thr Leu Thr
 275 280 285
Phe Val Tyr Leu Tyr Asn Ala Ala Ile Ser Leu Gly Tyr Ala Asn Ser
 290 295 300
Cys Leu Asn Pro Phe Val Tyr Ile Val Leu Cys Glu Thr Phe Arg Lys
 305 310 315 320
Arg Leu Val Leu Ser Val Lys Pro Ala Ala Gln Gly Gln Leu Arg Thr
 325 330 335
Val Ser Asn Ala Gln Thr Ala Asp Glu Glu Arg Thr Glu Ser Lys Gly
 340 345 350
Thr Val Asp Gly Thr Ala Gly Pro Gly Ser Ile Ala Thr Met Val Ser
 355 360 365

Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu
 370 375 380
 Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu
 385 390 395 400
 Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr
 405 410 415
 Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Thr Leu Thr Tyr
 420 425 430
 Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp
 435 440 445
 Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile
 450 455 460
 Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe
 465 470 475 480
 Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe
 485 490 495
 Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn
 500 505 510
 Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys
 515 520 525
 Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu
 530 535 540
 Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu
 545 550 555 560
 Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp
 565 570 575
 Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala
 580 585 590
 Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
 595 600

<210> 12

<211> 592

<212> PRT

<213> Artificial Sequence

<220>

<223> Mouse MCH1R/EGFP

<400> 12
 Met Asp Leu Gln Ala Ser Leu Leu Ser Thr Gly Pro Asn Ala Ser Asn
 1 5 10 15
 Ile Ser Asp Gly Gln Asp Asn Phe Thr Leu Ala Gly Pro Pro Pro Arg
 20 25 30
 Thr Arg Ser Val Ser Tyr Ile Asn Ile Ile Met Pro Ser Val Phe Gly
 35 40 45
 Thr Ile Cys Leu Leu Gly Ile Val Gly Asn Ser Thr Val Ile Phe Ala
 50 55 60
 Val Val Lys Lys Ser Lys Leu His Trp Cys Ser Asn Val Pro Asp Ile
 65 70 75 80
 Phe Ile Ile Asn Leu Ser Val Val Asp Leu Leu Phe Leu Leu Gly Met
 85 90 95
 Pro Phe Met Ile His Gln Leu Met Gly Asn Gly Val Trp His Phe Gly
 100 105 110
 Glu Thr Met Cys Thr Leu Ile Thr Ala Met Asp Ala Asn Ser Gln Phe
 115 120 125
 Thr Ser Thr Tyr Ile Leu Thr Ala Met Ala Ile Asp Arg Tyr Leu Ala
 130 135 140
 Thr Val His Pro Ile Ser Ser Thr Lys Phe Arg Lys Pro Ser Met Ala
 145 150 155 160
 Thr Leu Val Ile Cys Leu Leu Trp Ala Leu Ser Phe Ile Ser Ile Thr
 165 170 175

Pro Val Trp Leu Tyr Ala Arg Leu Ile Pro Phe Pro Gly Gly Ala Val
 180 185 190
 Gly Cys Gly Ile Arg Leu Pro Asn Pro Asp Thr Asp Leu Tyr Trp Phe
 195 200 205
 Thr Leu Tyr Gln Phe Phe Leu Ala Phe Ala Leu Pro Phe Val Val Ile
 210 215 220
 Thr Ala Ala Tyr Val Lys Ile Leu Gln Arg Met Thr Ser Ser Val Ala
 225 230 235 240
 Pro Ala Ser Gln Arg Ser Ile Arg Leu Arg Thr Lys Arg Val Thr Arg
 245 250 255
 Thr Ala Ile Ala Ile Cys Leu Val Phe Phe Val Cys Trp Ala Pro Tyr
 260 265 270
 Tyr Val Leu Gln Leu Thr Gln Leu Ser Ile Ser Arg Pro Thr Leu Thr
 275 280 285
 Phe Val Tyr Leu Tyr Asn Ala Ala Ile Ser Leu Gly Tyr Ala Asn Ser
 290 295 300
 Cys Leu Asn Pro Phe Val Tyr Ile Val Leu Cys Glu Thr Phe Arg Lys
 305 310 315 320
 Arg Leu Val Leu Ser Val Lys Pro Ala Ala Gln Gly Gln Leu Arg Thr
 325 330 335
 Val Ser Asn Ala Gln Thr Ala Asp Glu Glu Arg Thr Glu Ser Lys Gly
 340 345 350
 Thr Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile
 355 360 365
 Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser
 370 375 380
 Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe
 385 390 395 400
 Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr
 405 410 415
 Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met
 420 425 430
 Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln
 435 440 445
 Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala
 450 455 460
 Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys
 465 470 475 480
 Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu
 485 490 495
 Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys
 500 505 510
 Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly
 515 520 525
 Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp
 530 535 540
 Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala
 545 550 555 560
 Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu
 565 570 575
 Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
 580 585 590

<210> 13

<211> 604

<212> PRT

<213> Artificial Sequence

<220>

<223> MCH1R-linker-EGFP

<400> 13

Met Asp Leu Glu Ala Ser Leu Leu Pro Thr Gly Pro Asn Ala Ser Asn
 1 5 10 15
 Thr Ser Asp Gly Pro Asp Asn Leu Thr Ser Ala Gly Ser Pro Pro Arg
 20 25 30
 Thr Gly Ser Ile Ser Tyr Ile Asn Ile Ile Met Pro Ser Val Phe Gly
 35 40 45
 Thr Ile Cys Leu Leu Gly Ile Ile Gly Asn Ser Thr Val Ile Phe Ala
 50 55 60
 Val Val Lys Lys Ser Lys Leu His Trp Cys Asn Asn Val Pro Asp Ile
 65 70 75 80
 Phe Ile Ile Asn Leu Ser Val Val Asp Leu Leu Phe Leu Leu Gly Met
 85 90 95
 Pro Phe Met Ile His Gln Leu Met Gly Asn Gly Val Trp His Phe Gly
 100 105 110
 Glu Thr Met Cys Thr Leu Ile Thr Ala Met Asp Ala Asn Ser Gln Phe
 115 120 125
 Thr Ser Thr Tyr Ile Leu Thr Ala Met Ala Ile Asp Arg Tyr Leu Ala
 130 135 140
 Thr Val His Pro Ile Ser Ser Thr Lys Phe Arg Lys Pro Ser Met Ala
 145 150 155 160
 Thr Leu Val Ile Cys Leu Leu Trp Ala Leu Ser Phe Ile Ser Ile Thr
 165 170 175
 Pro Val Trp Leu Tyr Ala Arg Leu Ile Pro Phe Pro Gly Gly Ala Val
 180 185 190
 Gly Cys Gly Ile Arg Leu Pro Asn Pro Asp Thr Asp Leu Tyr Trp Phe
 195 200 205
 Thr Leu Tyr Gln Phe Phe Leu Ala Phe Ala Leu Pro Phe Val Val Ile
 210 215 220
 Thr Ala Ala Tyr Val Lys Ile Leu Gln Arg Met Thr Ser Ser Val Ala
 225 230 235 240
 Pro Ala Ser Gln Arg Ser Ile Arg Leu Arg Thr Lys Arg Val Thr Arg
 245 250 255
 Thr Ala Ile Ala Ile Cys Leu Val Phe Phe Val Cys Trp Ala Pro Tyr
 260 265 270
 Tyr Val Leu Gln Leu Thr Gln Leu Ser Ile Ser Arg Pro Thr Leu Thr
 275 280 285
 Phe Val Tyr Leu Tyr Asn Ala Ala Ile Ser Leu Gly Tyr Ala Asn Ser
 290 295 300
 Cys Leu Asn Pro Phe Val Tyr Ile Val Leu Cys Glu Thr Phe Arg Lys
 305 310 315 320
 Arg Leu Val Leu Ser Val Lys Pro Ala Ala Gln Gly Gln Leu Arg Thr
 325 330 335
 Val Ser Asn Ala Gln Thr Ala Asp Glu Glu Arg Thr Glu Ser Lys Gly
 340 345 350
 Thr Val Asp Gly Thr Ala Gly Pro Gly Ser Ile Ala Thr Met Val Ser
 355 360 365
 Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro Ile Leu Val Glu Leu
 370 375 380
 Asp Gly Asp Val Asn Gly His Lys Phe Ser Val Ser Gly Glu Gly Glu
 385 390 395 400
 Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys Phe Ile Cys Thr Thr
 405 410 415
 Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val Thr Leu Thr Tyr
 420 425 430
 Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His Met Lys Gln His Asp
 435 440 445
 Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val Gln Glu Arg Thr Ile
 450 455 460
 Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg Ala Glu Val Lys Phe
 465 470 475 480
 Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu Lys Gly Ile Asp Phe
 485 490 495

Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu Glu Tyr Asn Tyr Asn
 500 505 510
 Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln Lys Asn Gly Ile Lys
 515 520 525
 Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp Gly Ser Val Gln Leu
 530 535 540
 Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly Asp Gly Pro Val Leu
 545 550 555 560
 Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser Ala Leu Ser Lys Asp
 565 570 575
 Pro Asn Glu Lys Arg Asp His Met Val Leu Leu Glu Phe Val Thr Ala
 580 585 590
 Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr Lys
 595 600

<210> 14

<211> 673

<212> PRT

<213> Artificial Sequence

<220>

<223> MCH1R-linker-EGFP

<400> 14

Met Ser Val Gly Ala Met Lys Lys Gly Val Gly Arg Ala Val Gly Leu
 1 5 10 15
 Gly Gly Gly Ser Gly Cys Gln Ala Thr Glu Glu Asp Pro Leu Pro Asn
 20 25 30
 Cys Gly Ala Cys Ala Pro Gly Gln Gly Gly Arg Arg Trp Arg Leu Pro
 35 40 45
 Gln Pro Ala Trp Val Glu Gly Ser Ser Ala Arg Leu Trp Glu Gln Ala
 50 55 60
 Thr Gly Thr Gly Trp Met Asp Leu Glu Ala Ser Leu Leu Pro Thr Gly
 65 70 75 80
 Pro Asn Ala Ser Asn Thr Ser Asp Gly Pro Asp Asn Leu Thr Ser Ala
 85 90 95
 Gly Ser Pro Pro Arg Thr Gly Ser Ile Ser Tyr Ile Asn Ile Ile Met
 100 105 110
 Pro Ser Val Phe Gly Thr Ile Cys Leu Leu Gly Ile Ile Gly Asn Ser
 115 120 125
 Thr Val Ile Phe Ala Val Val Lys Lys Ser Lys Leu His Trp Cys Asn
 130 135 140
 Asn Val Pro Asp Ile Phe Ile Ile Asn Leu Ser Val Val Asp Leu Leu
 145 150 155 160
 Phe Leu Leu Gly Met Pro Phe Met Ile His Gln Leu Met Gly Asn Gly
 165 170 175
 Val Trp His Phe Gly Glu Thr Met Cys Thr Leu Ile Thr Ala Met Asp
 180 185 190
 Ala Asn Ser Gln Phe Thr Ser Thr Tyr Ile Leu Thr Ala Met Ala Ile
 195 200 205
 Asp Arg Tyr Leu Ala Thr Val His Pro Ile Ser Ser Thr Lys Phe Arg
 210 215 220
 Lys Pro Ser Met Ala Thr Leu Val Ile Cys Leu Leu Trp Ala Leu Ser
 225 230 235 240
 Phe Ile Ser Ile Thr Pro Val Trp Leu Tyr Ala Arg Leu Ile Pro Phe
 245 250 255
 Pro Gly Gly Ala Val Gly Cys Gly Ile Arg Leu Pro Asn Pro Asp Thr
 260 265 270
 Asp Leu Tyr Trp Phe Thr Leu Tyr Gln Phe Phe Leu Ala Phe Ala Leu
 275 280 285
 Pro Phe Val Val Ile Thr Ala Ala Tyr Val Lys Ile Leu Gln Arg Met
 290 295 300

Thr Ser Ser Val Ala Pro Ala Ser Gln Arg Ser Ile Arg Leu Arg Thr
 305 310 315 320
 Lys Arg Val Thr Arg Thr Ala Ile Ala Ile Cys Leu Val Phe Phe Val
 325 330 335
 Cys Trp Ala Pro Tyr Tyr Val Leu Gln Leu Thr Gln Leu Ser Ile Ser
 340 345 350
 Arg Pro Thr Leu Thr Phe Val Tyr Leu Tyr Asn Ala Ala Ile Ser Leu
 355 360 365
 Gly Tyr Ala Asn Ser Cys Leu Asn Pro Phe Val Tyr Ile Val Leu Cys
 370 375 380
 Glu Thr Phe Arg Lys Arg Leu Val Leu Ser Val Lys Pro Ala Ala Gln
 385 390 395 400
 Gly Gln Leu Arg Thr Val Ser Asn Ala Gln Thr Ala Asp Glu Glu Arg
 405 410 415
 Thr Glu Ser Lys Gly Thr Val Asp Gly Thr Ala Gly Pro Gly Ser Ile
 420 425 430
 Ala Thr Met Val Ser Lys Gly Glu Glu Leu Phe Thr Gly Val Val Pro
 435 440 445
 Ile Leu Val Glu Leu Asp Gly Asp Val Asn Gly His Lys Phe Ser Val
 450 455 460
 Ser Gly Glu Gly Glu Gly Asp Ala Thr Tyr Gly Lys Leu Thr Leu Lys
 465 470 475 480
 Phe Ile Cys Thr Thr Gly Lys Leu Pro Val Pro Trp Pro Thr Leu Val
 485 490 495
 Thr Thr Leu Thr Tyr Gly Val Gln Cys Phe Ser Arg Tyr Pro Asp His
 500 505 510
 Met Lys Gln His Asp Phe Phe Lys Ser Ala Met Pro Glu Gly Tyr Val
 515 520 525
 Gln Glu Arg Thr Ile Phe Phe Lys Asp Asp Gly Asn Tyr Lys Thr Arg
 530 535 540
 Ala Glu Val Lys Phe Glu Gly Asp Thr Leu Val Asn Arg Ile Glu Leu
 545 550 555 560
 Lys Gly Ile Asp Phe Lys Glu Asp Gly Asn Ile Leu Gly His Lys Leu
 565 570 575
 Glu Tyr Asn Tyr Asn Ser His Asn Val Tyr Ile Met Ala Asp Lys Gln
 580 585 590
 Lys Asn Gly Ile Lys Val Asn Phe Lys Ile Arg His Asn Ile Glu Asp
 595 600 605
 Gly Ser Val Gln Leu Ala Asp His Tyr Gln Gln Asn Thr Pro Ile Gly
 610 615 620
 Asp Gly Pro Val Leu Leu Pro Asp Asn His Tyr Leu Ser Thr Gln Ser
 625 630 635 640
 Ala Leu Ser Lys Asp Pro Asn Glu Lys Arg Asp His Met Val Leu Leu
 645 650 655
 Glu Phe Val Thr Ala Ala Gly Ile Thr Leu Gly Met Asp Glu Leu Tyr
 660 665 670
 Lys

<210> 15
 <211> 1269
 <212> DNA
 <213> Human

<400> 15
 atgtcagtgg gagccatgaa gaagggagtg gggagggcag ttgggcttgg aggcggcagc 60
 ggctgccagg ctacggagga agaccccctt cccaactgcg gggcttcgc tccgggacaa 120
 ggtggcaggc gctggaggct gccgcagcct gcgtgggtgg aggggagctc agctcggttg 180
 tgggagcagg cgaccggcac tggctggatg gacctggaaag cctcgctgct gcccactgg 240
 cccaacgcac gcaaacacctc tgatggcccc gataacctca cttccggcagg atcacctcct 300
 cgcacgggaa gcatctccta catcaacatc atcatgcctt cggtgttcgg caccatctgc 360
 ctccctggca tcatacgaa ctcacggtc atcttcgcgg tcgtgaagaa gtccaaagctg 420

cactggtgca	acaacgtccc	cgacatcttc	atcatcaacc	tctcggtagt	agatctcctc	480
tttccctgg	gcatgccctt	catgatccac	cagctcatgg	gcaatgggggt	gtggcacttt	540
ggggagacca	tgtgcaccc	catcacggcc	atggatgcca	atagtcagtt	caccaggacc	600
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<223> n = A,T,C or G						

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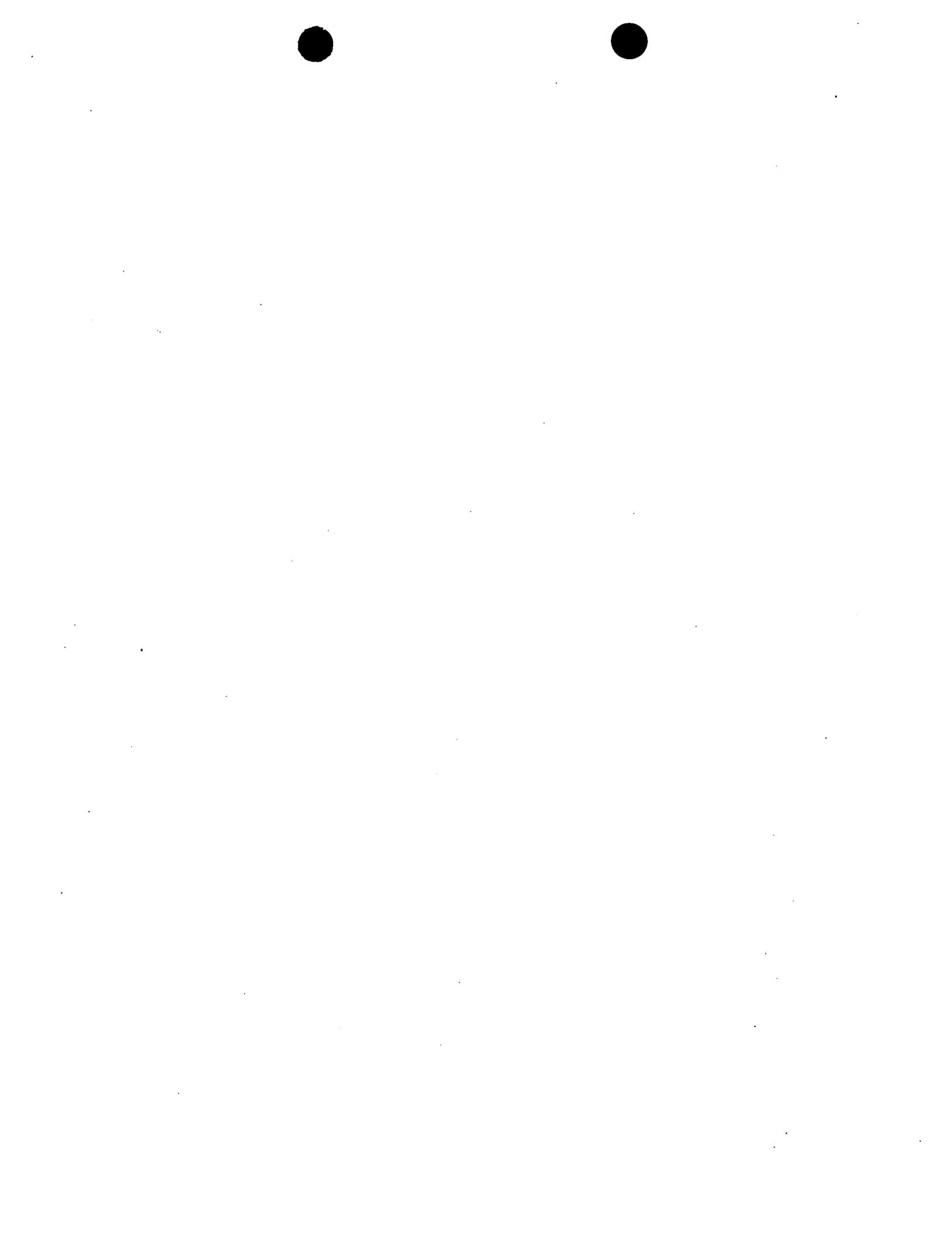
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<211> 3357
<212> DNA
<213> Mouse

<220>
<221> misc_feature
<222> (1)...(3357)
<223> n = A,T,C or G

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<210> 19

<211> 1062

<212> DNA

<213> Artificial Sequence

<220>

<223> Human short form/mouse species chimeric MCH1R

<400> 19

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<211> 1269

<212> DNA
 <213> Artificial Sequence

<220>

<223> Human long form/mouse species chimeric MCH1R
 <400> 20

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<210> 21

<211> 966

<212> DNA

<213> Aequorea Victoria

<400> 21

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<210> 22

<211> 765

<212> DNA

<213> Artificial Sequence

<220>

<223> GFP derivative





<400> 22

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<210> 23

<211> 720

<212> DNA

<213> Artificial Sequence

<220>

<223> GFP derivative

<400> 23

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<210> 24

<211> 720

<212> DNA

<213> Artificial Sequence

<220>

<223> GFP derivative

<400> 24

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<210> 25

<211> 720

<212> DNA

<213> Artificial Sequence

<220>

<223> GFP derivative

<400> 25

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<210> 26

<211> 3092

<212> DNA

<213> Artificial Sequence

<220>

<223> Mouse MCH1R-linker-EGFP

<400> 26

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<210> 27

<211> 3056

<212> DNA

<213> Artificial Sequence

<220>

<223> Mouse MCH1R/EGFP direct fusion

<400> 27

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<210> 28

<211> 1815

<212> DNA

<213> Artificial Sequence

<220>

<223> Human short form/mouse species chimeric
MCH1R-linker-EGFP

<400> 28

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<210> 29

<211> 2022

<212> DNA

<213> Artificial Sequence

<220>

<223> Human long form/mouse species chimeric
MCH1R-linker-EGFP

<400> 29

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<210> 30

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Linker

<400> 30

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<210> 31

<211> 12

<212> PRT

<213> Artificial Sequence

<220>

<223> Linker

<400> 31
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<210> 32
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> PCR primer

<400> 32
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<212> DNA
<213> Artificial Sequence

<220>
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<210> 34
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<220>
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<220>
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<220>

<223> PCR primer

<400> 37

gctctagagt cgcgccgct tacttgtaca gctcggtcc

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/08071

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07K 14/72 , 19/00; C12N 15/62
 US CL : 435/69.7, 252.3, 320.1; 530/350; 536/23.4

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/69.7, 252.3, 320.1; 530/350; 536/23.4

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN, MEDLINE
 search terms: fluores?, receptor#, green, g protein, melanin concentrating hormone receptor#

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NELSON et al. Characterization of an Intrinsically Fluorescent Gonadotropin-Releasing Hormone Receptor and Effects of Ligand Binding on Receptor Lateral Diffusion. Endocrinology. February 1999. Vol. 140. No. 2. pages 950-957, see entire document.	1-30
Y	AWAJI et al. Real-Time Optical Monitoring of Ligand-Mediated Internalization of alpha1b-Adrenoreceptor with Green Fluorescent Protein. Molecular Endocrinology. August 1998. Vol. 12. No. 8. pages 1099-1111, see entire document.	1-30

 Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

21 JUNE 2001

Date of mailing of the international search report

03 JUL 2001

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US01/08071

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	BACHNER et al. Identification of Melanin Concentrating Hormone (MCH) as the Natural Ligand for the Orphan Somatostatin-Like Receptor 1 (SLC-1). FEBS Letters. 03 September 1999. Vol. 457. No.3. pages 522-524, see entire document.	1-30
Y	SALRO et al. Molecular Characterization of the Melanin-Concentrating-Hormone Receptor. Nature. 15 July 1999. Vol. 400. pages 265-269, see entire document.	1-30